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Patents ADP number (if you know it)

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If the applicant is a corporate body, give the
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4. Title of the invention

HUMAN ANTIBODY MOLECULES

5. Name of your agent (if you have one)

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60/487,512

15 JULY 2003

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HUMAN ANTIBODY MOLECULES

The present invention relates to specific binding members, in particular human anti-IL-13 antibody molecules and especially those which neutralise IL-13 activity. It further relates to methods for using anti-IL-13 antibody molecules in diagnosis or treatment of IL-13 related disorders, including asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

10

Preferred embodiments of the present invention employ the antibody VH and/or VL domain of the antibody molecule herein termed BAK502G9 and other antibody molecules of the BAK502G9 lineage and of the BAK278D6 lineage, as herein defined.

15 Further preferred embodiments employ complementarity determining regions (CDRs) of the BAK278D6 lineage, and preferably BAK502G9, especially VH CDR3 in other antibody framework regions. Further aspects of the present invention provide for compositions containing specific binding members 20 of the invention, and their use in methods of inhibiting or neutralising IL-13, including methods of treatment of the human or animal body by therapy.

25 The present invention provides antibody molecules of particular value in binding and neutralising IL-13, and thus of use in any of a variety of therapeutic treatments, as indicated by the experimentation contained herein and further by the supporting technical literature.

30 Interleukin (IL)-13 is a 114 amino acid cytokine with an unmodified molecular mass of approximately 12 kDa [1,2]. IL-13 is most closely related to IL-4 with which it shares 30% sequence similarity at the amino acid level. The human IL-13 gene is located on chromosome 5q31 adjacent to the IL-4 gene

[1][2]. This region of chromosome 5q contains gene sequences for other Th2 lymphocyte derived cytokines including GM-CSF and IL-5, whose levels together with IL-4 have been shown to correlate with disease severity in asthmatics and rodent models of allergic inflammation [3][4][5][6][7][8].

Although initially identified as a Th2 CD4+ lymphocyte derived cytokine, IL-13 is also produced by Th1 CD4+ T-cells, CD8+ T lymphocytes NK cells, and non-T-cell populations such as mast cells, basophils, eosinophils, macrophages, monocytes and airway smooth muscle cells.

IL-13 is reported to mediate its effects through a receptor system that includes the IL-4 receptor α chain (IL-4R α), which 15 itself can bind IL-4 but not IL-13, and at least two other cell surface proteins, IL-13R α 1 and IL-13R α 2 [9][10]. IL-13R α 1 can bind IL-13 with low affinity, subsequently recruiting IL-4R α to form a high affinity functional receptor that signals [11][12]. The Genbank database lists the amino acid sequence 20 and the nucleic acid sequence of IL-13R α 1 as NP_001551 and Y10659 respectively. Studies in STAT6 (signal transducer and activator of transcription 6) -deficient mice have revealed that IL-13, in a manner similar to IL-4, signals by utilising the JAK-STAT6 pathway [13][14]. IL-13R α 2 shares 37% sequence 25 identity with IL-13R α 1 at the amino acid level and binds IL-13 with high affinity [15][16]. However, IL-13R α 2 has a shorter cytoplasmic tail that lacks known signalling motifs. Cells expressing IL-13R α 2 are not responsive to IL-13 even in the presence of IL-4R α [17]. It is postulated, therefore, that IL-30 13R α 2 acts as a decoy receptor regulating IL-13 but not IL-4 function. This is supported by studies in IL-13R α 2 deficient mice whose phenotype was consistent with increased responsiveness to IL-13 [18][19]. The Genbank database lists

the amino acid sequence and the nucleic acid sequence of IL-13R α 2 as NP_000631 and Y08768 respectively.

5 The signalling IL-13R α 1/IL-4R α receptor complex is expressed on human B-cells, mast cells, monocyte/macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells and airway smooth muscle cells.

10 Bronchial asthma is a common persistent inflammatory disease of the lung characterised by airways hyper-responsiveness, mucus overproduction, fibrosis and raised serum IgE levels. Airways hyper-responsiveness (AHR) is the exaggerated constriction of the airways to non-specific stimuli such as cold air. Both AHR and mucus overproduction are thought to be 15 responsible for the variable airway obstruction that leads to the shortness of breath characteristic of asthma attacks (exacerbations) and which is responsible for the mortality associated with this disease (around 2000 deaths/year in the United Kingdom).

20

The incidence of asthma, along with other allergic diseases, has increased significantly in recent years [20][21]. For example, currently, around 10% of the population of the United Kingdom (UK) has been diagnosed as asthmatic.

25

Current British Thoracic Society (BTS) and Global Initiative for Asthma (GINA) guidelines suggest a stepwise approach to the treatment of asthma [22, 23]. Mild to moderate asthma can usually be controlled by the use of inhaled corticosteroids, 30 in combination with beta-agonists or leukotriene inhibitors. However, due to the documented side effects of corticosteroids, patients tend not to comply with the treatment regime which reduces the effectiveness of treatment [24-26].

There is a clear need for new treatments for subjects with more severe disease, who often gain very limited benefit from either higher doses of inhaled or oral corticosteroids

5 recommended by asthma guidelines. Long term treatment with oral corticosteroids is associated with side effects such as osteoporosis, slowed growth rates in children, diabetes and oral candiditis [88]. As both beneficial and adverse effects of corticosteroids are mediated via the same receptor, 10 treatment is a balance between safety and efficacy.

Hospitalisation of these patients, who represent around 6% of the UK asthma population, as a result of severe exacerbations accounts for the majority of the significant economic burden 15 of asthma on healthcare authorities [89].

15 It is believed that the pathology of asthma is caused by ongoing Th2 lymphocyte mediated inflammation that results from inappropriate responses of the immune system to harmless antigens. Evidence has been accrued which implicates IL-13, 20 rather than the classical Th2 derived cytokine IL-4, as the key mediator in the pathogenesis of established airway disease.

25 Administration of recombinant IL-13 to the airways of naïve non-sensitised rodents caused many aspects of the asthma phenotype including airway inflammation, mucus production and AHR [27] [28] [29] [30]. A similar phenotype was observed in a transgenic mouse in which IL-13 was specifically overexpressed in the lung. In this model more chronic exposure to IL-13 also 30 resulted in fibrosis [31].

Further, in rodent models of allergic disease many aspects of the asthma phenotype have been associated with IL-13. Soluble murine IL-13Ra2, a potent IL-13 neutraliser, has been shown to

inhibit AHR, mucus hypersecretion and the influx of inflammatory cells which are characteristics of this rodent model [27] [28] [30]. In complementary studies, mice in which the IL-13 gene had been deleted, failed to develop allergen-induced AHR. AHR could be restored in these IL-13 deficient mice by the administration of recombinant IL-13. In contrast, IL-4 deficient mice developed airway disease in this model [32] [33].

10 Using a longer-term allergen-induced pulmonary inflammation model, Taube et al. demonstrated the efficacy of soluble murine IL-13R α 2 against established airway disease [34]. Soluble murine IL-13R α 2 inhibited AHR, mucus overproduction and to a lesser extent airway inflammation. In contrast, 15 soluble IL-4R α , which binds and antagonises IL-4, had little effect on AHR or airway inflammation in this system [35]. These findings were supported by Bleasdale et al. who developed a chronic fungal model of asthma in which polyclonal antibodies against IL-13 but not IL-4 were able to reduce mucus 20 overproduction, AHR and subepithelial fibrosis [36].

A number of genetic polymorphisms in the IL-13 gene have also been linked to allergic disease. In particular, a variant of the IL-13 gene in which the arginine residue at amino acid 130 25 is substituted with glutamine (Q130R) has been associated with bronchial asthma, atopic dermatitis and raised serum IgE levels [37] [38] [39] [40]. This particular IL-13 variant is also referred to as the Q110R variant (arginine residue at amino acid 110 is substituted with glutamine) by some groups who 30 exclude the 20 amino acid signal sequence from the amino acid count. Arima et al, [41] report that this variant is associated with raised levels of IL-13 in serum. The IL-13 variant (Q130R) and antibodies to this variant are discussed in WO 01/62933. An IL-13 promoter polymorphism, which alters

IL-13 production, has also been associated with allergic asthma [42].

Raised levels of IL-13 have also been measured in human subjects with asthma, atopic rhinitis (hay fever), allergic dermatitis (eczema) and chronic sinusitis. For example levels of IL-13 were found to be higher in bronchial biopsies, sputum and broncho-alveolar lavage (BAL) cells from asthmatics compared to control subjects [43][44][45][46]. Further, levels of IL-13 in BAL samples increased in asthmatic individuals upon challenge with allergen [47][48]. The IL-13 production capacity of CD4(+) T cells has further been shown to be useful marker of risk for subsequent development of allergic disease in newborns [49].

Li et al [114] have recently reported affects of a neutralising anti-mouse IL-13 antibody in a chronic mouse model of asthma. Chronic asthma-like response (such as AHR, sever airway inflammation, hyper mucus productions) was induced in OVA sensitised mice. Li et al report that administration of an IL-13 antibody at the time of each OVA challenge suppresses AHR, eosinophil infiltration, serum IgE levels, proinflammatory cytokine/chemokine levels and airway remodelling [14].

IL-13 may play a role in the pathogenesis of inflammatory bowel disease. Heller et al.[116] report that neutralisation of IL-13 by administration of soluble IL-13R α 2 ameliorated colonic inflammation in a murine model of human ulcerative colitis [116]. Correspondingly, IL-13 expression was higher in rectal biopsy specimens from ulcerative colitis patients when compared to controls [117].

In summary, these data provide indication that IL-13 rather than IL-4 is a more attractive target for the treatment of human allergic disease.

5 Aside from asthma, IL-13 has been associated with other fibrotic conditions. Increased levels of IL-13, up to a 1000 fold higher than IL-4, have been measured in the serum of patients with systemic sclerosis [50] and in BAL samples from patients affected with other forms of pulmonary fibrosis [51].

10 Correspondingly, overexpression of IL-13 but not IL-4 in the mouse lung resulted in pronounced fibrosis [52][53]. The contribution of IL-13 to fibrosis in tissues other than the lung has been extensively studied in a mouse model of parasite-induced liver fibrosis. Specific inhibition of IL-13

15 by administration of soluble IL-13Ra2 or IL-13 gene disruption, but not ablation of IL-4 production prevented fibrogenesis in the liver [54][55][56].

20 Chronic Obstructive Pulmonary Disease (COPD) includes patient populations with varying degrees of chronic bronchitis, small airway disease and emphysema and is characterised by progressive irreversible lung function decline that responds poorly to current asthma based therapy [90].

25 The incidence of COPD has risen dramatically in recent years to become the fourth leading cause of death worldwide (World Health Organisation). COPD therefore represents a large unmet medical need.

30 The underlying causes of COPD remain poorly understood. The "Dutch hypothesis" proposes that there is a common susceptibility to COPD and asthma and therefore, that similar mechanisms may contribute to the pathogenesis of both disorders [57].

Zheng et al [58] have demonstrated that overexpression of IL-13 in the mouse lung caused emphysema, elevated mucus production and inflammation, reflecting aspects of human COPD. Furthermore, AHR, an IL-13 dependent response in murine models of allergic inflammation, has been shown to be predictive of lung function decline in smokers [59]. A link has also been established between an IL-13 promoter polymorphism and susceptibility to develop COPD [60].

10 The signs are therefore that IL-13 plays an important role in the pathogenesis of COPD, particularly in patients with asthma-like features including AHR and eosinophilia. mRNA levels of IL-13 have been shown to be higher in autopsy tissue samples from subjects with a history of COPD when compared to 15 lung samples from subjects with no reported lung disease (J. Elias, Oral communication at American Thoracic Society Annual Meeting 2002). In another study, raised levels of IL-13 were demonstrated by immunohistochemistry in peripheral lung sections from COPD patients [91].

20 Hodgkin's disease is a common type of lymphoma, which accounts for approximately 7,500 cases per year in the United States. Hodgkin's disease is unusual among malignancies in that the neoplastic Reed-Sternberg cell, often derived from B-cells, 25 make up only a small proportion of the clinically detectable mass. Hodgkin's disease-derived cell lines and primary Reed-Sternberg cells frequently express IL-13 and its receptor [61]. As IL-13 promotes cell survival and proliferation in normal B-cells, it was proposed that IL-13 could act as a 30 growth factor for Reed-Sternberg cells. Skinnider et al. have demonstrated that neutralising antibodies against IL-13 can inhibit the growth of Hodgkin's disease-derived cell lines *in vitro* [62]. This finding suggested that Reed-Sternberg cells might enhance their own survival by an IL-13 autocrine and

paracrine cytokine loop. Consistent with this hypothesis, raised levels of IL-13 have been detected in the serum of some Hodgkin's disease patients when compared to normal controls [63]. IL-13 inhibitors may therefore prevent disease

5 progression by inhibiting proliferation of malignant Reed-Sternberg cells.

Many human cancer cells express immunogenic tumour specific antigens. However, although many tumours spontaneously

10 regress, a number evade the immune system (immunosurveillance) by suppressing T-cell mediated immunity. Terabe et al. [64] have demonstrated a role of IL-13 in immunosuppression in a mouse model in which tumours spontaneously regress after initial growth and then recur. Specific inhibition of IL-13, 15 with soluble IL-13Ra2, protected these mice from tumour recurrence. Terabe et al [64] went on to show that IL-13 suppresses the differentiation of tumour specific CD8+ cytotoxic lymphocytes that mediate anti-tumour immune responses.

20

IL-13 inhibitors may, therefore, be used therapeutically to prevent tumour recurrence or metastasis. Inhibition of IL-13 has been shown to enhance anti-viral vaccines in animal models and may be beneficial in the treatment of HIV and other 25 infectious diseases [65].

It should be noted that generally herein reference to interleukin-13 or IL-13 is, except where context dictates otherwise, reference to human IL-13. This is also referred to

30 in places as "the antigen". The present invention provides antibodies to human IL-13, especially human antibodies, that are cross-reactive with non-human primate IL-13, including cynomolgus and rhesus monkey IL-13. Antibodies in accordance with some embodiments of the present invention recognise a

variant of IL-13 in which the arginine residue at amino acid position 130 is replaced by glutamine. In other aspects and embodiments the present invention provides specific binding members against murine IL-13, specifically mouse IL-13.

5

Brief Description of Figures

Figure 1 shows neutralisation potency (% inhibition) of BAK167A11 (closed squares) and its derivative BAK615E3 (open squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

15. Figure 2 shows the neutralisation potency (% inhibition) of BAK278D6 (closed squares) and its derivative BAK502G9 (open squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate 20 determinations within the same experiment.

Figure 3 shows the neutralisation potency (% inhibition) of BAK209B11 (closed squares) as a scFv against 25 ng/ml murine IL-13 in the TF-1 cell proliferation assay. The triangles. 25 represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

30. Figure 4 shows the neutralisation potency (% inhibition) of BAK278D6 (closed squares) as a scFv against IL-13 in the TF-1 cell proliferation assay. The triangles represent irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 4A show potency against 25 ng/ml human IL-13.

Figure 4B shows potency against 25 ng/ml human variant IL-13.

Figure 4C shows potency against 50 ng/ml non-human primate IL-13.

5

Figure 5 shows a comparison of the potency of anti-human IL-13 antibodies in the TF-1 proliferation assay. Data represent the mean neutralisation potency with standard error bars over 5-7 experiments against 25 ng/ml human IL-13. The performance relative to the commercially available antibody, B-B13, was evaluated statistically by performing a one-way ANOVA with Dunnett's test. * P<0.05, ** P<0.01 compared to B-B13.

Figure 6 shows the neutralisation potency (% inhibition) of BAK502G9 (closed squares), BAK1167F2 (closed triangles) and BAK1183H4 (closed inverted triangles) as human IgG4 against tagged IL-13 in the TF-1 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of three separate experiments.

Figure 6A shows potency against 25 ng/ml human IL-13.

Figure 6B shows potency against 25 ng/ml human variant IL-13.

Figure 6C shows potency against 50 ng/ml non-human primate IL-13.

25

Figure 7 shows the neutralisation potency (% inhibition) of BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 and commercial anti-human IL-13 antibodies (B-B13 - open squares; JES10-5A2 - open inverted triangles) in the native IL-13 dependent HDLM-2 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 8 shows a comparison of the potency of anti-human IL-13 antibodies in the NHLF assay. Data represent the mean neutralisation potency (IC_{50} pM) with standard error bars over 5 4-5 experiments against 10 ng/ml human IL-13 in the NHLF eotaxin release assay. The performance relative to the commercially available antibody, B-B13, was evaluated statistically by performing a one-way ANOVA with Dunnett's test. * $P<0.05$, ** $P<0.01$ compared to B-B13.

10

Figure 9 shows the neutralisation potency (% inhibition) of BAK502G9 (closed triangles), BAK1167F2 (closed inverted triangles), BAK1183H4 (diamonds) as human IgG4 against VCAM-1 upregulation on the surface of HUVEC in response to 10ng/ml 15 human IL-13. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 10 shows the neutralisation potency (% inhibition) of BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 against eotaxin release from VCAM-1 upregulation on the surface of HUVEC in response to either 1ng/ml human IL-4 (Figure 10A) or 25 0.5ng/ml human IL-1 β (Figure 10B). Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 11 shows the neutralisation potency (% inhibition) of BAK209B11 (squares) as a human IgG4 against 1 ng/ml murine IL-30 13 in the factor dependent B9 cell proliferation assay. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 12 shows the relative level of IL-13 in lung homogenates from sensitised (s) (right-hand bar) and non-sensitised (ns) (left-hand bar) mice post challenge in a murine model of acute pulmonary allergic inflammation. The effect of sensitisation was statistically evaluated by performing Student's t-test using quantity of IL-13 data. * <0.05 . ** <0.01 compared to non-sensitised control animals (n=5-6 mice). Data represent the mean with standard error bars.

Figure 13 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced leukocyte recruitment to the lung in ovalbumin sensitised mice. The number of leukocytes is shown ($\times 10^4$). The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

Figure 14 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced eosinophil recruitment to the lung in ovalbumin sensitised mice. The number of eosinophils is shown ($\times 10^4$). The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

Figure 15 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin

induced neutrophil recruitment to the lung in ovalbumin sensitised mice. The number of neutrophils is shown ($\times 10^4$). The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

Figure 16 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced lymphocyte recruitment to the lung in ovalbumin sensitised mice. The induction of lymphocytes was dose dependently inhibited by BAK209B11 with maximal inhibition at 3 μ g/ml of BAK209B11. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to ovalbumin challenged PBS control animals (=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

Figure 17 illustrates the effects of i.v. administration of BAK209B11 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on ovalbumin induced monocyte/macrophage recruitment to the lung in ovalbumin sensitised mice. There was no significant increase in the levels of monocytes/macrophages of sensitised animals when compared with control animals. However, such background levels of these cells were depressed by $\geq 36\mu$ g/ml BAK209B11 in sensitised animals. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to ovalbumin challenged PBS control animals

(=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

5 Figure 18 shows the effects of a commercial anti-IL-13 neutralising antibody JES10-5A2 on the influx of cells (number of leukocytes is shown ($\times 10^4$)) into the murine airpouch elicited by administration of bacterially derived recombinant human IL-13. The effect of antibody treatment was

10 statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. * <0.05 . ** <0.01 compared to CMC control animals (=0% inhibition; n=11-13 mice). Data represent the mean with standard error bars.

15 Figure 19 shows an sequence alignment of cynomolgus IL-13 against human IL-13 amino acid sequences. The seven amino acid residues that differ between human and cynomolgus IL-13 are shaded. Rhesus and cynomolgus IL-13 have an identical amino acid sequence.

20

Figure 20 illustrates the effects of single 10mg/kg i.v bolus dose of BAK502G9 as human IgG4 on serum IgE levels in 4 allergic but non-challenged cynomolgus primates' (2 male/2 female) over 29 days. Serum IgE concentration is significantly

25 reduced from 100 % (predose) to $66 \pm 10\%$ of control values ($p<0.05$), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovers to $88 \pm 8\%$ of control levels by day 22. * = $p<0.05$ as compared to predose IgE levels, repeated measures ANOVA followed by Dunnett's multiple

30 comparisons test (n=4 animals).

Figure 21 illustrates the effects of intraperitoneal administration of BAK209B11 in different amounts (H=237 μ g/day, M=23.7 μ g/day and L=2.37 μ g/day) compared with an isotype

matched IgG1 irrelevant control antibody on the lung function of ovalbumin sensitised and challenged mice. In Figure 21A lung function is represented by log PC₅₀s (log methacholine concentration required to increase baseline PenH by 50%) before any treatment (day 0) and post sensitisation, challenge and drug treatment (day 25). Figure 21A shows the raw data used to calculate the study endpoint, shown in figure 21B (Delta log PC₅₀). Data represent the mean with standard error bars of n=8.

10

In Figure 21B changing lung function is shown by a change in an individual mouse's log PC₅₀ (delta log PC₅₀). Delta log PC₅₀ is defined as an individual's change in log PC₅₀ at day 25 versus day 0. Data represent group mean delta log PC₅₀ (individual changes averaged within treatment groups) with standard error bars. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using delta log PC₅₀ data. **p<0.01 compared to ovalbumin sensitised and challenged control animals (n=8 mice).

20

Figure 22 illustrates the effects of local (i.p.) and systemic (i.v.) administration of BAK502G9 as human IgG4 in different amounts compared to an isotype matched IgG4 irrelevant control antibody on the total leukocyte recruitment (Figure 22A) and eosinophil recruitment (Figure 22B) into the air pouch of BALB/C mice. Data represent the mean with standard error bars of n=10. The effect of antibody treatment was statistically evaluated by performing one way ANOVA with Dunnett's test using log-transformed data. *p<0.05, **p<0.01 compared to huIL-13 challenged mice (n=10).

In various aspects and embodiments of the invention there is provided the subject-matter of the claims included below.

The present invention provides specific binding members for IL-13, in particular human and/or primate IL-13 and/or variant IL-13 (Q130R), and murine IL-13. Preferred embodiments within 5 the present invention are antibody molecules, whether whole antibody (e.g. IgG, such as IgG4) or antibody fragments (e.g. scFv, Fab, dAb). Antibody antigen binding regions are provided, as are antibody VH and VL domains. Within VH and VL domains are provided complementarity determining regions, 10 CDR's, which may be provided within different framework regions, FR's, to form VH or VL domains as the case may be. An antigen binding site may consist of an antibody VH domain and/or a VL domain.

15 An antigen binding site may be provided by means of arrangement of CDR's on non-antibody protein scaffolds such as fibronectin or cytochrome B etc. [115, 116]. Scaffolds for engineering novel binding sites in proteins have been reviewed in detail by Nygren et al [116]. Protein scaffolds for 20 antibody mimics are disclosed in WO/0034784 in which the inventors describe proteins (antibody mimics) which include a fibronectin type III domain having at least one randomised loop. A suitable scaffold into which to graft one or more CDR's, e.g. a set of HCDR's, may be provided by any domain 25 member of the immunoglobulin gene superfamily.

Preferred embodiments of the present invention are in what is termed herein the "BAK278D6 lineage". This is defined with reference to a set of six CDR sequences of BAK278D6 as 30 follows: HCDR1 (SEQ ID NO: 1), HCDR2 (SEQ ID NO: 2), HCDR3 (SEQ ID NO: 3), LCDR1 (SEQ ID NO: 4), LCDR2 (SEQ ID NO: 5) and LCDR3 (SEQ ID NO: 6). In one aspect, the present invention provides a specific binding member for human IL-13, comprising an antibody antigen-binding site which is composed of a human

antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the HCDR1 has the amino acid sequence of 5 SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6; or wherein 10 the set of CDR's contains one or two amino acid substitutions compared with the set of CDR's, wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid 15 sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6.

The set of CDR's wherein the HCDR1 has the amino acid sequence 20 of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6, are herein 25 referred to as the "BAK278D6 set of CDR's". The HCDR1, HCDR2 and HCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of HCDR's" and the LCDR1, LCDR2 and LCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of LCDR's". A set of CDR's with the BAK278D6 30 set of CDR's, BAK278D6 set of HCDR's or BAK278D6 LCDR's, or one or two substitutions therein, is said to be of the BAK278D6 lineage.

As noted, in one aspect the invention provides a specific binding member for human IL-13, comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a 5 set of CDR's, wherein the set of CDR's is the BAK278D6 set of CDR's or a set of CDR's containing one or two substitutions compared with the BAK278D6 set of CDR's.

10 In preferred embodiments, the one or two substitutions are at one or two of the following residues within the CDRs of the VH and/or VL domains, using the standard numbering of Kabat [107].

31, 32, 34 in HCDR1

15

52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

20 26, 27, 28, 30, 31 in LCDR1

56 in LCDR2

95A, 97 in LCDR3

25

Preferred embodiments have two substitutions compared with the BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27. Of these embodiments, preferred embodiments have S substituted for N at HCDR3 residue 99 and/or I substituted for 30 N at LCDR1 residue 27. Still further embodiments have a substitution at HCDR3 residue 99 selected from the group consisting of S, A, I, R, P and K, and/or a substitution at LCDR1 residue 27 selected from the group consisting of I, L, M, C, V, K, Y, F, R, T, S, A, H and G.

In preferred embodiments one or two substitutions are made at one or two of the following residues within the BAK278D6 set of CDR's in accordance with the identified groups of possible 5 substitute residues:

	<u>Position of substitution</u>	<u>Substitute Residue selected from the group consisting of</u>
10	31 in HCDR1:	Q, D, L, G and E
	32 in HCDR1:	T
	34 in HCDR1:	V, I and F
15	52 in HCDR2:	D, N, A, R, G and E
	52A in HCDR2:	D, G, T, P, N and Y
	53 in HCDR2:	D, L, A, P, T, S, I and R
	54 in HCDR2:	S, T, D, G, K and I
20	56 in HCDR2:	T, E, Q, L, Y, N, V, A, M and G
	58 in HCDR2:	I, L, Q, S, M, H, D and K
	60 in HCDR2:	R
	61 in HCDR2:	R
25	62 in HCDR2:	K and G
	64 in HCDR2:	R
	65 in HCDR2:	K
	96 in HCDR3:	R and D
30	97 in HCDR3:	N, D, T and P
	98 in HCDR3:	R
	99 in HCDR3:	S, A, I, R, P and K
	101 in HCDR3:	Y

26 in LCDR1: D and S
27 in LCDR1: I, L, M, C, V, K, Y, F, R, T, S, A, H and G
28 in LCDR1: V
30 in LCDR1: G
5 31 in LCDR1: R

56 in LCDR2: T

95A in LCDR3: N
10 97 in LCDR3: I

Preferred embodiments have the BAK278D6 set of CDR's with a substitution of S for N at residue 99 within HCDR3 and I for N at residue 27 within LCDR 1. The set of CDR's thus defined is 15 as follows: HCDR1 - SEQ ID NO: 7; HCDR2 - SEQ ID NO: 8, HCDR3 - SEQ ID NO: 9; LCDR1 - SEQ ID NO: 10, LCDR2 - SEQ ID NO: 11; LCDR3 - SEQ ID NO: 12. This set of CDR's is herein referred to as the "BAK502G9 set of CDR's".

20 Further preferred embodiments have the BAK278D6 set of CDR's with one or two substitutions within the CDR's, with the proviso that the pair of substitutions of S for N at residue 99 within HCDR3 and I for N at residue 27 within LCDR 1 is excluded.

25 Other preferred embodiments are as follows: BAK 1166G2: HCDR1- SEQ ID NO: 67, HCDR2- SEQ ID NO: 68, HCDR3- SEQ ID NO: 69, LCDR1 - SEQ ID NO: 70, LCDR2 - SEQ ID NO: 71; LCDR3 - SEQ ID NO: 72.

30 BAK1167F2 HCDR1- SEQ ID NO: 61, HCDR2- SEQ ID NO: 62, HCDR3- SEQ ID NO: 63, LCDR1 - SEQ ID NO: 64, LCDR2 - SEQ ID NO: 65; LCDR3 - SEQ ID NO: 66.

BAK1184C8: HCDR1- SEQ ID NO:73, HCDR2: SEQ ID NO:74, HCDR3- SEQ ID NO:75. LCDR1 - SEQ ID NO: 76, LCDR2 - SEQ ID NO: 77; LCDR3 - SEQ ID NO: 78.

5 BAK1185E1: HCDR1- SEQ ID NO:79, HCDR2- SEQ ID NO:80, HCDR3- SEQ ID NO: 81. LCDR1 - SEQ ID NO: 82, LCDR2 - SEQ ID NO: 83; LCDR3 - SEQ ID NO: 84.

10 BAK1167F4: HCDR1- SEQ ID NO: 85, HCDR2- SEQ ID NO:86, HCDR3- SEQ ID NO:87. LCDR1 - SEQ ID NO: 88, LCDR2 - SEQ ID NO: 89; LCDR3 - SEQ ID NO: 90.

15 BAK1111D10: HCDR1- SEQ ID NO: 91, HCDR2- SEQ ID NO: 92, HCDR3- SEQ ID NO: 93. LCDR1 - SEQ ID NO: 94, LCDR2 - SEQ ID NO: 95; LCDR3 - SEQ ID NO: 96.

20 BAK1183H4: HCDR1- SEQ ID NO: 97, HCDR2- SEQ ID NO: 98, HCDR3- SEQ ID NO: 99. LCDR1 - SEQ ID NO: 100, LCDR2 - SEQ ID NO: 101; LCDR3 - SEQ ID NO: 102.

25 BAK1185F8: HCDR1- SEQ ID NO: 103, HCDR2- SEQ ID NO: 104, HCDR3- SEQ ID NO: 105. LCDR1 - SEQ ID NO: 106, LCDR2 - SEQ ID NO: 107; LCDR3 - SEQ ID NO: 108. All of these were derived from 502G9 by heavy chain CDR1 and CDR2 randomisation and are thus of the BAK502G9 lineage.

30 A VH domain comprising a set of CDR's HCDR1, HCDR2 and HCDR3 of any clone as shown in Table 1. Table 1 is also provided by the present invention, as is separately a VL domain comprising a set of CDR's LCDR1, LCDR2 and LCDR3 of the clones shown in Table 1. Preferably such a VH domain is paired with such a VL domain, and most preferably the VH and VL domain pairings are the same as in the clones as set out in Table 1.

Further provided by the present invention is a VH domain comprising a set of CDR's HCDR1, HCDR2 and HCDR3 wherein the set of CDR's corresponds to that for any clone shown in Table 1 with one or two amino acid substitutions.

5 Further provided by the present invention is a VL domain comprising a set of CDR's LCDR1, LCDR2 and LCDR3 wherein the set of CDR's corresponds to that for any clone shown in Table 1 with one or two amino acid substitutions.

10 A specific binding member comprising an antibody antigen-binding domain comprising such a VH and/or VL domain is also provided by the present invention.

15 The present inventors have identified the BAK278D6 lineage as providing human antibody antigen-binding domains against IL-13 which are of particular value. Within the lineage, BAK502G9 has been identified to be of special value. The BAK278D6 and BAK502G9 sets of CDR's have been identified already above.

20 Following the lead of computational chemistry in applying multivariate data analysis techniques to the structure/property-activity relationships [94], quantitative activity-property relationships of antibodies can be derived 25 using well-known mathematical techniques such as statistical regression, pattern recognition and classification [95-100]. The properties of antibodies can be derived from empirical and theoretical models (for example, analysis of likely contact residues or calculated physicochemical property) of antibody 30 sequence, functional and three-dimensional structures and these properties can be considered singly and in combination.

An antibody antigen-binding site composed of a VH domain and a VL domain is formed by six loops of polypeptide: three from

the light chain variable domain (VL) and three from the heavy chain variable domain (VH). Analysis of antibodies of known atomic structure has elucidated relationships between the sequence and three-dimensional structure of antibody combining sites[101,102]. These relationships imply that, except for the third region (loop) in VH domains, binding site loops have one of a small number of main-chain conformations: canonical structures. The canonical structure formed in a particular loop has been shown to be determined by its size and the presence of certain residues at key sites in both the loop and in framework regions [101,102].

This study of sequence-structure relationship can be used for prediction of those residues in an antibody of known sequence, but of an unknown three-dimensional structure, which are important in maintaining the three-dimensional structure of its CDR loops and hence maintain binding specificity. These predictions can be backed up by comparison of the predictions to the output from lead optimization experiments.

In a structural approach, a model can be created of the antibody molecule [103] using a freely available or commercial package such as WAM [104]. A protein visualisation and analysis software package such as Insight II [105] or Deep View [106] may then be used to evaluate possible substitutions at each position in the CDR. This information may then be used to make substitutions likely to have a minimal or beneficial effect on activity.

The present inventors analysed sequence data of the panel of clones for which the sets of CDR's are shown in Table 1.

The analysis tested the hypothesis that any binary combinations of listed amino acid variations in the CDR's from

the presented set of scFv variants leads to a scFv variant with at least the starting potency of the parent scFv BAK278D6.

5 All scFv variants in the panel shown in Table 1 have been selected for improved affinity and have been confirmed to display higher potency.

10 The observed amino acid variations can either be favourable, non-favourable or neutral in their effect on the starting potency of scFv BAK278D6 in the TF-1 assay of 44nM.

15 No linkage was observed between any two amino acid variations confirming that there was no synergy, either "positive" or "negative", between any two selected amino acid variations.

20 There are four scenarios where such binary combination will fulfil the hypothesis and three scenarios where the hypothesis will not be valid. Synergistic amino acid variants are not considered as no linkage was observed.

The hypothesis is valid where:

A1: mutation 1 is favourable and mutation 2 is favourable

25 A2 : mutation 1 is favourable and mutation 2 is neutral

A3: mutation 1 is neutral and mutation 2 is neutral

A4: mutation 1 is favourable and mutation 2 is non-favourable (with the effect of 1 outweighing the effect of 2) .

30 The hypothesis is not valid where:

B1: mutation 1 is non-favourable and mutation 2 is neutral

B2: mutation 1 is non-favourable and mutation 2 is non-favourable

B3: mutation 1 is favourable and mutation 2 is non-favourable (with the effect of 2 outweighing the effect of 1).

For A4 to be possible, mutation 1 needs to be highly favourable to counterbalance the negative effect of mutation 2 on potency. Since such highly favourable mutation would be present in the library of variants used for selection, it would be selected for and would therefore appear frequently in the panel of variants. Since synergy can be excluded, such mutation would be beneficial in any kind of sequence context and should therefore reappear in different scFv variants. An example for such frequent amino acid change is the change in the light chain CDR1 Asn27Ile. However, this mutation on its own (in clone BAK531E2) has only a modest 2-fold effect on potency (final IC50 of 23.2nM). On its own this mutation would not allow the scenario depicted in A4, as it is not a highly favourable mutation. This suggests that every clone in the presented set of IL-13 binding clones (Table 1) which has a light chain CDR1 Asn27Ile change along with one or more further mutations is at least as potent as the variant having the single light chain CDR1 Asn27Ile mutation. The other mutations are either neutral or positive but do not have a negative or detrimental affect.

A further example is in the heavy chain CDR3 Asn99Ser (see Table 1). As a clone carrying this particular single amino acid variation is not observed, the potency of such a clone has been estimated to be approximately 12.0nM by the following rationale:

BAK278D6 potency is 44nM. Alterations of VL CDR1 N27I + VH CDR3 N99S lead to BAK502G9 with potency 8nM, i.e. 5.5 fold improvement.

BAK278D6 potency is 44nM. Alteration of VL CDR1 N27I leads to BAK531E2 with potency 23 nM, i.e. 1.9 fold improvement

BAK278D6 potency is 44nM. Alteration VH CDR3 N99S to provide 5 a possible clone with potency 12.2nM, i.e. 2.9 fold improvement (5.5/1.9 = 2.9).

The binary combination of heavy chain CDR3 Asn99Ser with light 10 chain CDR1 Asn27Ile gives a scFv BAK0502G9 with a potency of 8nM. As synergy is excluded, the contribution of heavy chain CDR3 Asn99Ser change in BAK502G9 is therefore additive.

Therefore every clone in the presented set of IL-13 binding clones (Table 1) which has a heavy chain CDR3 AsnH99Ser change 15 along with one or more further mutations would have a potency of at least 12nM or greater.

Thus, the inventors note that a highly favourable amino acid variation which would be selected preferentially is not 20 observed. As discussed above, two variations which were prominently represented in Table 1 of scFv variants were analysed closer. Any scFv variant in Table 1 with either of these mutations along with one or more further mutations displayed a potency which was at least as improved as a clone 25 containing any one of these two single amino acid variations in the parent BAK278D6. There is therefore no evidence that a highly favourable amino acid variation, that would allow scenario A4, is present in the panel.

30 This observation led the inventors to conclude that there were no non-favourable mutations present in this set of scFv variants. This means scenarios A4 and B1 to B3 are not relevant and the hypothesis is valid.

Accordingly, as noted already, the present invention provides specific binding members comprising the defined sets of CDR's, in particular the set of CDR's of BAK278D6, and sets of CDR's of the BAK278D6 lineage, with one or two substitutions within 5 the set of CDR's, e.g. the BAK502G9 set of CDR's.

The relevant set of CDR's is provided within antibody framework regions or other protein scaffold, e.g. fibronectin or cytochrome B [115, 116]. Preferably antibody framework 10 regions are employed, and where they are employed they are preferably germline, more preferably the antibody framework region for the heavy chain may be DP14 from the VH1 family. The preferred framework region for the light chain may be λ 3-3H. For the BAK502G9 set of CDR's it is preferred that the 15 antibody framework regions are for VH FR1, SEQ ID NO: 27, for VH FR2, SEQ ID NO: 28, for VH FR3, SEQ ID NO 29, for light chain FR1, SEQ ID NO: 30, for light chain FR2, SEQ ID NO: 31, for light chain FR3, SEQ ID NO: 32. In a highly preferred embodiment, a VH domain is provided with the amino acid 20 sequence of SEQ ID NO: 15, this being termed "BAK502G9 VH domain". In a further highly preferred embodiment, a VL domain is provided with the amino acid sequence of SEQ ID NO: 16, this being termed "BAK502G9 VL domain". A highly preferred antibody antigen-binding site provided in accordance 25 with the present invention is composed of the BAK502G9 VH domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO: 16. This antibody antigen-binding site may be provided within any desired antibody molecule format, e.g. scFv, Fab, IgG, IgG4, dAb etc., as is discussed further elsewhere herein.

30

In a further highly preferred embodiment, the present invention provides an IgG4 antibody molecule comprising the BAK502G9 VH domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO: 16. This is termed herein "BAK502G9 IgG4".

Other IgG4 or other antibody molecules comprising the BAK502G9 VH domain, SEQ ID NO: 15, and/or the BAK502G9 VL domain, SEQ ID NO: 16, are provided by the present invention, as are other 5 antibody molecules comprising the BAK502G9 set of HCDR's (SEQ ID NO: 7, 8 and 9) within an antibody VH domain, and/or the BAK502G9 set of LCDR's (SEQ ID NO: 10, 11 and 12) within an antibody VL domain.

10 It is convenient to point out here that "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as 15 if each is set out individually herein.

As noted, the present invention provides a specific binding member which binds human IL-13 and which comprises the BAK502G9 VH domain (SEQ ID NO: 15) and/or the BAK502G9 VL 20 domain (SEQ ID NO: 16).

Generally, a VH domain is paired with a VL domain to provide an antibody antigen binding site, although as discussed further below a VH domain alone may be used to bind antigen. 25 In one preferred embodiment, the BAK502G9 VH domain (SEQ ID NO: 15) is paired with the BAK502G9 VL domain (SEQ ID NO: 16), so that an antibody antigen binding site is formed comprising both the BAK502G9 VH and VL domains. In other embodiments, the BAK502G9 VH is paired with a VL domain other than the 30 BAK502G9 VL. Light-chain promiscuity is well established in the art.

Similarly, any set of HCDR's of the BAK278D6 lineage can be provided in a VH domain that is used as a specific binding

member alone or in combination with a VL domain. A VH domain may be provided with a set of HCDR's of a BAK278D6 lineage antibody, e.g. as shown in Table 1, and if such a VH domain is paired with a VL domain, then the VL domain may be provided 5 with a set of LCDR's of a BAK278D6 lineage antibody, e.g. as shown in Table 1. A pairing of a set of HCDR's and a set of LCDR's may be as shown in Table 1, providing an antibody antigen-binding site comprising a set of CDR's as shown in Table 1. The framework regions of the VH and/or VL domains 10 may be germline frameworks. Framework regions of the heavy chain domain may be selected from the VH-1 family, and a preferred VH-1 framework is DP-14 framework. Framework regions of the light chain may be selected from the λ 3 family, and a preferred such framework is λ 3 3H.

15 One or more CDRs may be taken from the BAK502G9 VH or VL domain and incorporated into a suitable framework. This is discussed further herein. BAK502G9 HCDR's 1, 2 and 3 are shown in SEQ ID NO: 7, 8 and 9, respectively. BAK502G9 LCDR's 20 1, 2 and 3 are shown in SEQ ID NO: 10, 11 and 12, respectively.

The same applies for other BAK278D6 lineage CDR's and sets of CDR's as shown in Table 1.

25 Further embodiments of the invention relate to a specific binding member comprising the VH and/or VL domain, or an antigen binding site comprising CDRs of the VH and/or VL domain of the antibody molecule disclosed herein as 30 167A11 (VH: SEQ ID NO: 23 and VL: SEQ ID NO: 24) and its derivatives 615E3 (VH:SEQ ID NO: 33 and VL: SEQ ID NO: 34) BAK582F7 (VH CDR's SEQ ID's 141-143) and BAK612B5 (VH CDR's SEQ ID's 147-149). These recognise human IL-13. The derivatives of 167A11 from VH CDR3 randomisation are potent

scFv molecules (5-6nM). The 167A11 lineage may be employed in any aspect and embodiment of the present invention as disclosed herein for other molecules, for instance methods of mutation and selection of antigen binding sites with improved 5 potency.

Variants of the VH and VL domains and CDRs of the present invention, including those for which amino acid sequences are set out herein, and which can be employed in specific binding 10 members for IL-13 can be obtained by means of methods of sequence alteration or mutation and screening. Such methods are also provided by the present invention.

Variable domain amino acid sequence variants of any of the VH 15 and VL domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), may 20 be less than about 20 alterations, less than about 15 alterations, less than about 10 alterations or less than about 5 alterations, 4, 3, 2 or 1. Alterations may be made in one or more framework regions and/or one or more CDR's.

25 In accordance with further aspects of the present invention there is provided a specific binding member which competes for binding to antigen with any specific binding member which both binds the antigen and comprises a specific binding member, VH and/or VL domain disclosed herein, or HCDR3 disclosed herein, 30 or variant of any of these. Competition between binding members may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of

specific binding members which bind the same epitope or an overlapping epitope.

Thus, a further aspect of the present invention provides a 5 specific binding member comprising a human antibody antigen-binding site which competes with a BAK502G9 antibody molecule, in particular BAK502G9 scFv and/or IgG4, for binding to IL-13. In further aspects the present invention provides a specific 10 binding member comprising a human antibody antigen-binding site which competes with an antibody antigen-binding site for binding to IL-13, wherein the antibody antigen-binding site is composed of a VH domain and a VL domain, and wherein the VH and VL domains comprise a set of CDR's of the BAK278D6 lineage.

15 Various methods are available in the art for obtaining antibodies against IL-13 and which may compete with a BAK502G9 antibody molecule, an antibody molecule with a BAK502G9 set of CDR's, or an antibody molecule with a set of CDR's of BAK278D6 20 lineage, for binding to IL-13.

In a further aspect, the present invention provides a method of obtaining one or more specific binding members able to bind the antigen, the method including bringing into contact a 25 library of specific binding members according to the invention and said antigen, and selecting one or more specific binding members of the library able to bind said antigen.

The library may be displayed on the surface of bacteriophage 30 particles, each particle containing nucleic acid encoding the antibody VH variable domain displayed on its surface, and optionally also a displayed VL domain if present.

Following selection of specific binding members able to bind the antigen and displayed on bacteriophage particles, nucleic acid may be taken from a bacteriophage particle displaying a said selected specific binding member. Such nucleic acid may

5 be used in subsequent production of a specific binding member or an antibody VH variable domain (optionally an antibody VL variable domain) by expression from nucleic acid with the sequence of nucleic acid taken from a bacteriophage particle displaying a said selected specific binding member.

10

An antibody VH variable domain with the amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in isolated form, as may a specific binding member comprising such a VH domain.

15 Ability to bind IL-13 may be further tested, also ability to compete with BAK502G9 (e.g. in scFv format and/or IgG format, e.g. IgG4) for binding to IL-13. Ability to neutralise IL-13 may be tested, as discussed further below.

20 A specific binding member according to the present invention may bind IL-13 with the affinity of a BAK502G9 antibody molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with an affinity that is better.

25 A specific binding member according to the present invention may neutralise IL-13 with the potency of a BAK502G9 antibody molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with a potency that is better.

30 A specific binding member according to the present invention may neutralise naturally occurring IL-13 with the potency of a BAK502G9 antibody molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with a potency that is better.

Binding affinity and neutralisation potency of different specific binding members can be compared under appropriate conditions.

5 The antibodies of the present invention have a number of advantages over existing commercial anti-IL-13 antibodies, in particular three commercial rodent anti-human IL-13 antibodies namely, JES10-5A2 (BioSource), B-B13 (Euroclone) and clone 321166 (R&D Systems). The potency of the antibodies of the
10 present invention was compared with commercial antibodies JES10-A2 and B-B13. Clone 321166 was not evaluated as previous experiments revealed that this clone was considerably less potent than other known commercial antibodies.

15 The efficacy and use of the rodent commercial IL-13 antibodies in man is likely to be limited, because of their increased potential to induce immunogenic responses and therefore more rapid clearance from the body. Kinetic analysis of the antibodies of the present invention in non-human primates
20 suggests that these antibodies have a clearance rate which is similar to that of other known human or humanised antibodies.

Antibodies provided by various embodiments of the present invention recognize non-human primate IL-13, including rhesus
25 and cynomolgus IL-13. Determining efficacy and safety profiles of an antibody in non-human primates is extremely valuable as it provides a means for predicting the antibody's safety, pharmacokinetic and pharmacodynamic profile in humans.

30 Moreover, antibodies of various embodiments of the present invention further recognize the human IL-13 variant, Q130R, which is associated with asthma. Cross reactivity with variant IL-13 allows antibodies of the present invention and compositions comprising antibodies of the present invention to

be used for the treatment of patients with wild-type and variant IL-13.

A preferred embodiment of the present invention comprises 5 antibodies that neutralise naturally occurring IL-13 with a potency that is equal to or better than the potency of a IL-13 antigen binding site formed by BAK502G9 VH domain (SEQ ID NO:15) and the BAK502G9 VL domain (SEQ ID NO: 16). For example, the inventors have demonstrated that representative 10 clones such as BAK502G9, 1167F2 and 1183H4 are significantly more potent against naturally occurring IL-13 than known commercial antibodies (Figure 7).

In addition to antibody sequences, a specific binding member 15 according to the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Specific binding members of the invention may carry a 20 detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker).

In further aspects, the invention provides an isolated nucleic 25 acid which comprises a sequence encoding a specific binding member, VH domain and/or VL domains according to the present invention, and methods of preparing a specific binding member, a VH domain and/or a VL domain of the invention, which comprise expressing said nucleic acid under conditions to 30 bring about production of said specific binding member, VH domain and/or VL domain, and recovering it.

Specific binding members according to the invention may be used in a method of treatment or diagnosis of the human or

animal body, such as a method of treatment (which may include prophylactic treatment) of a disease or disorder in a human patient which comprises administering to said patient an effective amount of a specific binding member of the invention. Conditions treatable in accordance with the present invention include any in which IL-13 plays a role, especially asthma, atopic dermatitis, allergic rhinitis, fibrosis, chronic obstructive pulmonary disease, scleroderma, inflammatory bowel disease and Hodgkin's lymphoma. Further, the antibodies of the present invention may also be used in treating tumours and viral infections as these antibodies will inhibit IL-13 mediated immunosuppression [64, 65].

A further aspect of the present invention provides nucleic acid, generally isolated, encoding an antibody VH variable domain and/or VL variable domain disclosed herein.

Another aspect of the present invention provides nucleic acid, generally isolated, encoding a VH CDR or VL CDR sequence disclosed herein, especially a VH CDR selected from SEQ ID NO's: 7, 8 and 9 or a VL CDR selected from SEQ ID NO's: 10, 11 and 12, most preferably BAK502G9 VH CDR3 (SEQ ID NO: 9). Nucleic acid encoding the BAK502G9 set of CDR's, nucleic acid encoding the BAK502G9 set of HCDR's and nucleic acid encoding the BAK502G9 set of LCDR's are also provided by the present invention, as are nucleic acids encoding individual CDR's, HCDR's, LCDR's and sets of CDR's, HCDR's, LCDR's of the BAK278D6 lineage.

30 A further aspect provides a host cell transformed with nucleic acid of the invention.

A yet further aspect provides a method of production of an antibody VH variable domain, the method including causing

expression from encoding nucleic acid. Such a method may comprise culturing host cells under conditions for production of said antibody VH variable domain.

5 Analogous methods for production of VL variable domains and specific binding members comprising a VH and/or VL domain are provided as further aspects of the present invention.

10 A method of production may comprise a step of isolation and/or purification of the product.

15 A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.

20 These and other aspects of the invention are described in further detail below.

TERMINOLOGY

Specific binding member

This describes a member of a pair of molecules which have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or 25 partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have 30 the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. The present invention is concerned with antigen-antibody type reactions.

Antibody molecule

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any

5 polypeptide or protein comprising an antibody binding domain.

Antibody fragments which comprise an antigen binding domain are molecules such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use 10 techniques of recombinant DNA technology to produce other

antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve

introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an

15 antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for

instance, EP-A-184187, GB 2188638A or EP-A-239400, and a large body of subsequent literature. A hybridoma or other cell

producing an antibody may be subject to genetic mutation or

20 other changes, which may or may not alter the binding

specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody molecule" should be construed as covering any

25 specific binding member or substance having an antibody antigen-binding domain with the required specificity. Thus,

this term covers antibody fragments and derivatives, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric

30 molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore

included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023, and a large body

of subsequent literature.

Further techniques available in the art of antibody engineering have made it possible to isolate human and humanised antibodies. For example, human hybridomas can be 5 made as described by Kontermann et al [107]. Phage display, another established technique for generating specific binding members has been described in detail in many publications such as Kontermann et al [107] and WO92/01047 (discussed further below). Transgenic mice in which the mouse antibody genes are 10 inactivated and functionally replaced with human antibody genes while leaving intact other components of the mouse immune system, can be used for isolating human antibodies to human antigens [108].

15 Synthetic antibody molecules may be created by expression from genes generated by means of oligonucleotides synthesized and assembled within suitable expression vectors, for example as described by Knappik et al. J. Mol. Biol. (2000) 296, 57-86 or Krebs et al. Journal of Immunological Methods 254 2001 67-84.

20 It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and 25 CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989), McCafferty et al (1990) Nature, 348, 552-554) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent 30 fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-

5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al, Proc. Natl. Acad. Sci. USA 90 5 6444-6448, 1993). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Y. Reiter et al, Nature Biotech, 14, 1239-1245, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (S. Hu et al, Cancer Res., 56, 10 3055-3061, 1996).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current 15 Opinion Biotechnol. 4, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Examples of bispecific antibodies include those of the BiTE™ technology in which the binding domains of two antibodies with different 20 specificity can be used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

25 Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E.coli*. Diabodies (and many other polypeptides such as antibody fragments) of 30 appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against IL-13, then a library can be made where the other arm is varied and an antibody of appropriate

specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al, Protein Eng., 9, 616-621, 1996).

5 *Antigen-binding domain*

This describes the part of an antibody molecule which comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of 10 the antigen, which part is termed an epitope. An antigen binding domain may be provided by one or more antibody variable domains (e.g. a so-called Fd antibody fragment consisting of a VH domain). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) 15 and an antibody heavy chain variable region (VH).

Specific

This may be used to refer to the situation in which one member of a specific binding pair will not show any significant 20 binding to molecules other than its specific binding partner(s). The term is also applicable where e.g. an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be 25 able to bind to the various antigens carrying the epitope.

Comprise

This is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

30

Isolated

This refers to the state in which specific binding members of the invention, or nucleic acid encoding such binding members, will generally be in accordance with the present invention.

Isolated members and isolated nucleic acid will be free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Specific binding members may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells, or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

Naturally occurring IL-13

This generally refers to a state in which the IL-13 protein or fragments thereof may occur. Naturally occurring IL-13 means IL-13 protein which is naturally produced by a cell, without prior introduction of encoding nucleic acid using recombinant technology. Thus, naturally occurring IL-13 may be as produced naturally by for example CD4+ T cells and/or as isolated from a mammal, e.g. human, non-human primate, rodent such as rat or mouse.

Recombinant IL-13

This refers to a state in which the IL-13 protein or fragments thereof may occur. Recombinant IL-13 means IL-13 protein or fragments thereof produced by recombinant DNA in a heterologous host. Recombinant IL-13 may differ from naturally occurring IL-13 by glycosylation.

Recombinant proteins expressed in prokaryotic bacterial expression systems are not glycosylated while those expressed in eukaryotic systems such as mammalian or insect cells are 5 glycosylated. Proteins expressed in insect cells however differ in glycosylation from proteins expressed in mammalian cells.

By "substantially as set out" it is meant that the relevant 10 CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, amino acid substitutions may be 15 made in the CDR and/or VH or VL domain.

The structure for carrying a CDR or a set of CDR's of the invention will generally be of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR 20 or set of CDR's is located at a location corresponding to the CDR or set of CDR's of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to (Kabat, E.A. et al, 25 Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (<http://immuno.bme.nwu.edu> or find "Kabat" using any search engine).

30 CDR's can also be carried by other scaffolds such as fibronectin or cytochrome B [115, 116].

Preferably, a CDR amino acid sequence substantially as set out herein is carried as a CDR in a human variable domain or a substantial portion thereof. The HCDR3 sequences substantially as set out herein represent preferred 5 embodiments of the present invention and it is preferred that each of these is carried as a HCDR3 in a human heavy chain variable domain or a substantial portion thereof.

Variable domains employed in the invention may be obtained 10 from any germ-line or rearranged human variable domain, or may be a synthetic variable domain based on consensus sequences of known human variable domains. A CDR sequence of the invention (e.g. CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. CDR3), using recombinant DNA 15 technology.

For example, Marks et al (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to 20 the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks et al further describe how this repertoire may be combined with a CDR3 of a particular 25 antibody. Using analogous techniques, the CDR3-derived sequences of the present invention may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific binding members of the 30 invention. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 or any of a subsequent large body of literature, including Kay, B.K., Winter, J., and McCafferty, J. (1996) *Phage Display of Peptides and Proteins: A Laboratory Manual*, San Diego:

Academic Press, so that suitable specific binding members may be selected. A repertoire may consist of from anything from 10⁴ individual members upwards, for example from 10⁶ to 10⁸ or 10¹⁰ members. Other suitable host systems include yeast
5 display, bacterial display, T7 display, ribosome display and so on.

Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (*Nature*, 1994, 370:389-391), who
10 describes the technique in relation to a β -lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions
15 carrying CDR-derived sequences of the invention using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram et al (1992, *Proc. Natl. Acad. Sci., USA*, 89:3576-3580), who used error-prone PCR. In
20 preferred embodiments one or two amino acid substitutions are made within a set of HCDR's and/or LCDR's.

Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed
25 by Barbas et al, (1994, *Proc. Natl. Acad. Sci., USA*, 91:3809-3813) and Schier et al (1996, *J. Mol. Biol.* 263:551-567).

All the above described techniques are known as such in the art and in themselves do not form part of the present
30 invention. The skilled person will be able to use such techniques to provide specific binding members of the invention using routine methodology in the art.

A further aspect of the invention provides a method for obtaining an antibody antigen binding domain specific for IL-13 antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more 5 amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a 10 specific binding member or an antibody antigen binding domain specific for IL-13 antigen and optionally with one or more preferred properties, preferably ability to neutralise IL-13 activity. Said VL domain may have an amino acid sequence which is substantially as set out herein.

15

An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

20 In a preferred embodiment, BAK502G9 VH domain (SEQ ID NO: 15) may be subject to mutation to provide one or more VH domain amino acid sequence variants, and/or BAK502G9 VL (SEQ ID NO: 16) .

25 A further aspect of the invention provides a method of preparing a specific binding member specific for IL-13 antigen, which method comprises:

- (a) providing a starting repertoire of nucleic acids encoding a VH domain which either include a CDR3 to be 30 replaced or lack a CDR3 encoding region;
- (b) combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out herein for a VH CDR3 such that said donor nucleic acid is inserted into the CDR3 region in the repertoire, so as to

provide a product repertoire of nucleic acids encoding a VH domain;

(c) expressing the nucleic acids of said product repertoire;

5 (d) selecting a specific binding member specific for a IL-13; and

(e) recovering said specific binding member or nucleic acid encoding it.

10 Again, an analogous method may be employed in which a VL CDR3 of the invention is combined with a repertoire of nucleic acids encoding a VL domain which either include a CDR3 to be replaced or lack a CDR3 encoding region.

15 Similarly, one or more, or all three CDRs may be grafted into a repertoire of VH or VL domains which are then screened for a specific binding member or specific binding members specific for IL-13.

20 In a preferred embodiment, one or more of BAK502G9 HCDR1 (SEQ ID NO: 7), HCDR2 (SEQ ID NO: 8) and HCDR3 (SEQ ID NO: 9), or the BAK502G9 set of HCDR's, may be employed, and/or one or more of BAK502G9 LCDR1 (SEQ ID NO: 10), LCDR2 (SEQ ID NO: 11), or the BAK502G9 set of LCDR's.

25 A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the 30 first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with

naturally occurring variable domain regions. For example, construction of specific binding members of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers 5 introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies) 10 or protein labels as discussed in more detail elsewhere herein.

Although in a preferred aspect of the invention specific binding members comprising a pair of VH and VL domains are 15 preferred, single binding domains based on either VH or VL domain sequences form further aspects of the invention. It is known that single immunoglobulin domains, especially VH domains, are capable of binding target antigens in a specific manner.

20 In the case of either of the single specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific binding member able to bind IL-13.

25 This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach as disclosed in WO92/01047, in which an individual colony containing either an H or L chain clone is used to infect a 30 complete library of clones encoding the other chain (L or H) and the resulting two-chain specific binding member is selected in accordance with phage display techniques such as those described in that reference. This technique is also disclosed in Marks *et al*, *ibid*.

Specific binding members of the present invention may further comprise antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to 5 antibody light chain constant domains including human C κ or C λ chains, preferably C λ chains. Similarly, a specific binding member based on a VH domain may be attached at its C-terminal end to all or part (e.g. a CH1 domain) of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, 10 IgE and IgM and any of the isotype sub-classes, particularly IgG1 and IgG4. IgG4 is preferred. IgG4 is preferred because it does not bind complement and does not create effector functions. Any synthetic or other constant region variant that has these properties and stabilizes variable regions is 15 also preferred for use in embodiments of the present invention.

Specific binding members of the invention may be labelled with a detectable or functional label. Detectable labels include 20 radiolabels such as ^{131}I or ^{99}Tc , which may be attached to antibodies of the invention using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin which may be detected via 25 binding to a specific cognate detectable moiety, e.g. labelled avidin.

Specific binding members of the present invention are designed to be used in methods of diagnosis or treatment in human or 30 animal subjects, preferably human.

Accordingly, further aspects of the invention provide methods of treatment comprising administration of a specific binding member as provided, pharmaceutical compositions comprising

such a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the 5 specific binding member with a pharmaceutically acceptable excipient.

Clinical indications in which an anti-IL-13 antibody may be used to provide therapeutic benefit include asthma, atopic 10 dermatitis, allergic rhinitis, fibrosis, chronic obstructive pulmonary disease, inflammatory bowel disease, scleroderma and Hodgkin's lymphoma . As already explained, anti-IL-13 treatment is effective for all these diseases.

15 Anti-IL-13 treatment may be given orally, by injection (for example, subcutaneously, intravenously, intraperitoneal or intramuscularly), by inhalation, or topically (for example intraocular, intranasal, rectal, into wounds, on skin). The route of administration can be determined by the 20 physicochemical characteristics of the treatment, by special considerations for the disease or by the requirement to optimise efficacy or to minimise side-effects.

It is envisaged that anti-IL-13 treatment will not be 25 restricted to use in the clinic. Therefore, subcutaneous injection using a needle free device is also preferred.

Combination treatments may be used to provide significant synergistic effects, particularly the combination of an anti- 30 IL-13 specific binding member with one or more other drugs. A specific binding member according to the present invention may be provided in combination or addition to short or long acting beta agonists, corticosteroids, cromoglycate, leukotriene (receptor) antagonists, methyl xanthines and their

derivatives, IL-4 inhibitors, muscarinic receptor antagonists, IgE inhibitors, histaminic inhibitors, IL-5 inhibitors, eotaxin/CCR3 inhibitors, PDE4 inhibitors, TGF-beta antagonists, interferon-gamma, perfenidone, chemotherapeutic 5 agents and immunotherapeutic agents.

Combination treatment with one or more short or long acting beta agonists, corticosteroids, cromoglycate, leukotriene (receptor) antagonists, xanthines, IgE inhibitors, IL-4 10 inhibitors, IL-5 inhibitors, eotaxin/CCR3 inhibitors, PDE4 inhibitors may be employed for treatment of asthma. Antibodies of the present invention can also be used in combination with corticosteroids, anti-metabolites, antagonists of TGF-beta and its downstream signalling pathway, for treatment of fibrosis. 15 Combination therapy of these antibodies with PDE4 inhibitors, xanthines and their derivatives, muscarinic receptor antagonists, short and long beta antagonists can be useful for treating chronic obstructive pulmonary disease. Similar consideration of combinations apply to the use of anti-IL-13 20 treatment for atopic dermatitis, allergic rhinitis, chronic obstructive pulmonary disease, inflammatory bowel disease, scleroderma and Hodgkin's lymphoma.

In accordance with the present invention, compositions 25 provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of 30 administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors. Appropriate doses of antibody are well known in the art; see Ledermann J.A. et al.

(1991) Int. J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922.

- 5 The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment or diabody), and the nature of any detectable label
- 10 or other molecule attached to the antibody. A typical antibody dose will be in the range 100 μ g to 1 gm for systemic applications, and 1 μ g to 1mg for topical applications. Typically, the antibody will be a whole antibody, preferably the IgG4 isotype. This is a dose for a single treatment of an
- 15 adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. In preferred embodiments
- 20 of the present invention, treatment is periodic, and the period between administrations is about two weeks or more, preferably about three weeks or more, more preferably about four weeks or more, or about once a month.
- 25 Specific binding members of the present invention will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the specific binding member.
- 30 Thus pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in

the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by

5 injection, e.g. intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid

10 pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may

15 be included.

For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free

20 and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants

25 and/or other additives may be included, as required.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

30

Specific binding members of the present invention may be formulated in liquid or solid forms depending on the physicochemical properties of the molecule and the route of delivery. Formulations may include excipients, or combinations

of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of antibody concentrations and pH. Solid formulations may be produced by lyophilisation, spray drying, or drying by 5 supercritical fluid technology, for example. Formulations of anti-IL-13 will depend upon the intended route of delivery: for example, formulations for pulmonary delivery may consist of particles with physical properties that ensure penetration into the deep lung upon inhalation; topical formulations may 10 include viscosity modifying agents, which prolong the time that the drug is resident at the site of action.

The present invention provides a method comprising causing or allowing binding of a specific binding member as provided 15 herein to IL-13. As noted, such binding may take place *in vivo*, e.g. following administration of a specific binding member, or nucleic acid encoding a specific binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation, affinity 20 chromatography, or cell based assays such as a TF-1 assay.

The amount of binding of specific binding member to IL-13 may be determined. Quantitation may be related to the amount of the antigen in a test sample, which may be of diagnostic 25 interest.

A kit comprising a specific binding member or antibody molecule according to any aspect or embodiment of the present invention is also provided as an aspect of the present invention. In a kit of the invention, the specific binding member or antibody molecule may be labelled to allow its reactivity in a sample to be determined, e.g. as described further below. Components of a kit are generally sterile and in sealed vials or other containers. Kits may be employed in

diagnostic analysis or other methods for which antibody molecules are useful. A kit may contain instructions for use of the components in a method, e.g. a method in accordance with the present invention. Ancillary materials to assist in 5 or to enable performing such a method may be included within a kit of the invention.

The reactivities of antibodies in a sample may be determined by any appropriate means. Radioimmunoassay (RIA) is one 10 possibility. Radioactive labelled antigen is mixed with unlabelled antigen (the test sample) and allowed to bind to the antibody. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the antibody determined. The more antigen there is in the 15 test sample the less radioactive antigen will bind to the antibody. A competitive binding assay may also be used with non-radioactive antigen, using antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor or laser dye with spectrally isolated 20 absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

25 Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or 30 otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral

absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

5

The signals generated by individual antibody-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

10

The present invention also provides the use of a specific binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a specific binding member as provided by the present invention in a competition assay. This may be where the physical separation of bound from unbound antigen is not required. Linking a reporter molecule to the specific binding member so that a physical or optical change occurs on binding is one possibility. The reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

The present invention also provides for measuring levels of antigen directly, by employing a specific binding member according to the invention for example in a biosensor system.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to

choose a suitable mode according to their preference and general knowledge.

As noted, in various aspects and embodiments, the present
5 invention extends to a specific binding member which competes
for binding to IL-13 with any specific binding member defined
herein, e.g. BAK502G9 IgG4. Competition between binding
members may be assayed easily *in vitro*, for example by tagging
a specific reporter molecule to one binding member which can
10 be detected in the presence of other untagged binding
member(s), to enable identification of specific binding
members which bind the same epitope or an overlapping epitope.

Competition may be determined for example using ELISA in which
15 IL-13 is immobilised to a plate and a first tagged binding
member along with one or more other untagged binding members
is added to the plate. Presence of an untagged binding member
that competes with the tagged binding member is observed by a
decrease in the signal emitted by the tagged binding member.

20 In testing for competition a peptide fragment of the antigen
may be employed, especially a peptide including an epitope of
interest. A peptide having the epitope sequence plus one or
more amino acids at either end may be used. Such a peptide
25 may be said to "consist essentially" of the specified
sequence. Specific binding members according to the present
invention may be such that their binding for antigen is
inhibited by a peptide with or including the sequence given.
In testing for this, a peptide with either sequence plus one
30 or more amino acids may be used.

Specific binding members which bind a specific peptide may be isolated for example from a phage display library by panning with the peptide(s).

5 The present invention further provides an isolated nucleic acid encoding a specific binding member of the present invention. Nucleic acid may include DNA and/or RNA. In a preferred aspect, the present invention provides a nucleic acid which codes for a CDR or set of CDR's or VH domain or VL
10 domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4, of the invention as defined above.

15 The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

20 The present invention also provides a recombinant host cell which comprises one or more constructs as above. A nucleic acid encoding any CDR or set of CDR's or VH domain or VL domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4 as provided, itself forms an aspect of the present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently
25 be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression a VH or VL domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

30

Specific binding members, VH and/or VL domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or

homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may 5 comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires 10 otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, plant cells, yeast 15 and baculovirus systems and transgenic plants and animals. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NS0 mouse melanoma cells, YB2/0 rat myeloma cells, human embryonic 20 kidney cells, human embryonic retina cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the 25 art. For a review, see for example Plückthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding member for example Chadd HE and Chamow SM (2001) 110 Current Opinion in 30 Biotechnology 12: 188-194, Andersen DC and Krummen L (2002) Current Opinion in Biotechnology 13: 117, Larrick JW and Thomas DW (2001) Current opinion in Biotechnology 12:411-418.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 3rd edition, Sambrook and Russell, 2001, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1988, 15 *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Ausubel et al. eds., John Wiley & Sons, 4th edition 1999. The disclosures of Sambrook et al. and Ausubel et al. (both) are incorporated herein by reference.

20 Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. Such a host cell may be *in vitro* and may be *in culture*. Such a host cell may be *in vivo*. *In vivo* presence of the host cell may 25 allow intracellular expression of the specific binding members of the present invention as "intrabodies" or intracellular antibodies. Intrabodies may be used for gene therapy [112].

30 A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using

retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. Introducing nucleic acid in the host cell, in particular a eukaryotic cell may use a viral or a plasmid based system. The plasmid system may be maintained episomally 5 or may incorporated into the host cell or into an artificial chromosome [110,111]. Incorporation may be either by random or targeted integration of one or more copies at single or multiple loci. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and 10 transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

15 In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with 20 standard techniques.

The present invention also provides a method which comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as 25 above.

Aspects and embodiments of the present invention will now be illustrated by way of example with reference to the following experimentation.

EXAMPLE 1***Isolation of anti-IL-13 scFv******ScFv antibody repertoire***

5 A large single chain Fv (scFv) human antibody library derived from spleen lymphocytes from 20 donors and cloned into a phagemid vector was used for selections [66].

Selection of scFv

10 ScFv which recognised IL-13 were isolated from phage display libraries in a series of repeated selection cycles on recombinant bacterially derived human or murine IL-13 (Peprotech) essentially as described in [67]. In brief, following incubation with the library, the immobilised 15 antigen, which had been pre-coupled to paramagnetic beads, and bound phage were recovered by magnetic separation whilst unbound phage were washed away. Bound phage was then rescued as described by Vaughan et al [67] and the selection process repeated. Different solid surfaces and capture methods were 20 used at different rounds of selection to reduce non-specific binding. Antigen was either covalently coupled to beads (Dynabeads M-270 carboxylic acid) or modified by biotinylation prior to secondary capture by streptavidin-coated beads (Dynabeads M-280) according to manufacturer's protocols 25 (Dynal). A representative proportion of clones from the output of selection rounds were subjected to DNA sequencing as described in Vaughan et al [67] and Osbourn et al [70]. Unique clones were assessed for their ability to neutralise IL-13 as purified scFv preparations in IL-13 dependent cell 30 proliferation assays.

Ribosome display libraries were created and screened for scFv that specifically recognised recombinant, bacterially derived human or murine IL-13 (Peprotech), essentially as described in

Hanes et al [113]. Initially the BAK278D6 lead clone from the initial selections was converted to ribosome display format, and this template was subsequently used for library creation. On the DNA level, a T7 promoter was added at the 5'-end for 5 efficient transcription to mRNA. On the mRNA level, the construct contained a prokaryotic ribosome-binding site (Shine-Dalgarno sequence). At the 3' end of the single chain, the stop codon was removed and a portion of gIII (gene III) was added to act as a spacer [113].

10

Ribosome display libraries derived from BAK278D6 were created by mutagenesis of antibody complementarity determining regions (CDRs) where PCR reactions were performed with non-proof reading Taq polymerase. Affinity-based selections were 15 performed whereby, following incubation with the library, the biotinylated human-IL-13 was captured by streptavidin-coated paramagnetic beads (Dynal M280) and bound tertiary complexes (mRNA-ribosome-scFv-IL-13) were recovered by magnetic separation whilst unbound complexes were washed away. The 20 mRNA encoding the bound scFvs were then recovered by RT-PCR as described in Hanes et al [113] and the selection process repeated with decreasing concentrations (100nM - 100pM over 5 rounds) of biotinylated human IL-13 present during the selection.

25

Error-prone PCR was also used to further increase library size. Three intensities of error were employed (2.0, 3.5 and 30 7.2 mutations per 1,000 bp after a standard PCR reaction, as described in manufacturer's protocol (Clontech)) during the selection regime. Initial error prone PCR reactions took place before round one selections commenced at 100nM. A subsequent round of error prone PCR was performed before round three selections at 10nM biotinylated human-IL-13. As above, a representative proportion of clones from the output of

selection rounds were subjected to DNA sequencing as described in Vaughan et al [67] and Osbourn et al [70]. Unique clones were assessed for their ability to neutralise IL-13 as purified scFv preparations in IL-13 dependent cell

5 proliferation assays.

EXAMPLE 2

Neutralisation potency of anti-IL-13 scFv in the IL-13 dependent TF-1 cell proliferation assay

10 The neutralisation potency of purified scFv preparations against human and murine IL-13 bioactivity was assessed using TF-1 cell proliferation assay. Purified scFv preparations were prepared as described in Example 3 of WO01/66754. Protein

15 concentrations of purified scFv preparations were determined using the BCA method (Pierce). TF-1 is a human premyeloid cell line established from a patient with erythroleukemia [68]. The TF-1 cell line is factor dependent for survival and proliferation. In this respect TF-1 cells responded to either

20 human or murine IL-13 [69] and were maintained in media containing human GM-CSF (4 ng/ml, R&D Systems). Inhibition of IL-13 dependent proliferation was determined by measuring the reduction in incorporation of tritiated thymidine into the newly synthesized DNA of dividing cells.

25

TF-1 cell assay protocol

TF-1 cells were obtained from R&D Systems and maintained according to supplied protocols. Assay media comprised RPMI-1640 with GLUTAMAX I (Invitrogen) containing 5% foetal bovine

30 serum (JRH) and 1% sodium pyruvate (Sigma). Prior to each assay, TF-1 cells were pelleted by centrifugation at 300 x g for 5 mins, the media removed by aspiration and the cells resuspended in assay media. This process was repeated twice with cells resuspended at a final concentration of 10^5 cells/ml

in assay media. Test solutions of antibody (in triplicate) were diluted to the desired concentration in assay media. An irrelevant antibody not directed at IL-13 was used as a negative control. Recombinant bacterially derived human or 5 murine IL-13 (Peprotech) was added to a final concentration of 50 ng/ml when mixed with the appropriate test antibody in a total volume of 100 μ l/well in a 96 well assay plate. The concentration of IL-13 used in the assay was selected as the dose that at final assay concentration gave approximately 80% 10 of the maximal proliferative response. All samples were incubated for 30 minutes at room temperature. 100 μ l of resuspended cells were then added to each assay point to give a total assay volume of 200 μ l/well. Assay plates were incubated for 72 hours at 37°C under 5% CO₂. 25 μ l of tritiated 15 thymidine (10 μ Ci/ml, NEN) was then added to each assay point and assay plates were returned to the incubator for a further 4 hours. Cells were harvested on glass fibre filter plates (Perkin Elmer) using a cell harvester. Thymidine incorporation was determined using a Packard TopCount microplate liquid 20 scintillation counter. Data were analysed using Graphpad Prism software.

Results

Despite alternating selection cycles between human and murine antigen no cross-reactive neutralising antibodies were obtained. Two distinct anti-human and one anti-murine IL-13 neutralising scFvs were obtained from selections. BAK278D6 (VH SEQ ID NO: 13; VL SEQ ID NO: 14) and BAK167A11 (VH SEQ ID NO: 23; VL SEQ ID NO: 24) recognised human IL-13 whilst BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) recognised murine IL-13. BAK278D6 (Figure 2) and BAK167A11 (Figure 1) as scFv 10 neutralised 25 ng/ml human IL-13 with an IC₅₀ of 44nM and 111nM respectively. BAK209B11 (Figure 3) as a scFv neutralised 25 ng/ml murine IL-13 with an IC₅₀ of 185nM.

EXAMPLE 3

15 *Neutralisation potency of lead clones from targeted optimisation of heavy chain CDR3 of parental clones in the IL-13 dependent TF-1 cell proliferation assay*

Osbourne et al. [70] have demonstrated that targeted 20 mutagenesis of residues within heavy chain CDR3 can significantly improve the affinity of antibodies. Selections were performed as described in Example 1, on scFv repertoires in which residues within the heavy chain CDR3 of BAK278D6 (SEQ ID NO: 6) BAK167A11 (SEQ ID NO: 57) had been randomised by 25 mutagenesis. Unique clones from the selection output were identified by DNA sequencing and their neutralising potency assessed as scFv in the TF-1 cell proliferation assay, as described in Example 2.

30 *Results*

Significant gains in potency were achieved for both lineages. The most potent clones from the BAK167A11 lineage were BAK615E3, BAK612B5 and BAK582F7 which as scFv had IC₅₀ of 3nM (Figure 1), 6.6nM, 6.65nM respectively against 25ng/ml human

IL-13 in TF-1 cell proliferation assay. From the BAK278D6 lineage, the most potent clone was BAK502G9, which as scFv had IC₅₀ of 8nM against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay (Figure 2).

5

EXAMPLE 4

Neutralisation potency of BAK167A11 and BAK278D6 lineages against non-human primate IL-13 and an IL-13 variant associated with asthma in the TF-1 factor dependent cell proliferation assay

Neither of the BAK167A11 and BAK278D6 human IL-13 neutralising lineages were murine cross-reactive. The inventors therefore decided on the following criteria for the lineage selected for 15 further optimisation and clinical development: should preferably be cross-reactive with non-human primate IL-13 and should recognise a variant of IL-13, in which arginine at amino acid at position 130 is substituted for by glutamine (Q130R). This variant has been genetically associated with 20 asthma and other allergic diseases [37, 39, 41, 71]. Cross-reactivity was determined by the ability of purified scFv preparations to bind non-human primate IL-13 and IL-13 variant by surface plasmon resonance (BIAcore) analysis. Functional activity was determined using the TF-1 cell proliferation 25 assay.

Production of wild-type, variant and non-human primate IL-13
A cDNA for wild-type human IL-13 was obtained from InvivoGen and modified by site-directed mutagenesis (Stratagene 30 Quikchange® kit) to yield a cDNA encoding variant IL-13. The coding sequence for both rhesus and cynomolgus monkey IL-13 was obtained by PCR on genomic DNA template using degenerate primers based on the human IL-13 sequence. Both non-human primate (rhesus and cynomolgus) sequences were identical to

each other but differed from human IL-13 by seven amino acids (Figure 19). Recombinant wild type, variant and non-human primate IL-13 were subsequently expressed using the baculovirus expression system (Invitrogen). Expression constructs added a carboxyl terminus affinity tag to the expressed protein that allowed purification from insect cell conditioned media to near homogeneity.

Qualitative binding assay using BIAcore

10 The binding affinity of purified scFv preparations to non-human primate, variant and wild type IL-13 was determined by surface plasmon resonance measurements using a BIAcore 2000 Biosensor (BIAcore AB) as described in Karlsson et al [72]. In brief, IL-13 was coupled to CM5 sensorchips using an amine 15 coupling kit (BIAcore) at a surface density of approximately 200Ru and three concentrations of test scFv (approximately 350nM, 175nM and 88nM) in HBS-EP buffer passed over the sensor chip surface. The resulting sensorgrams were evaluated using BIA evaluation 3.1 software to provide relative binding data.

20

TF-1 assay protocol

25 The assay was performed essentially as described in Example 2 with the following modifications: non-human primate IL-13, human variant IL-13 (130R) and wild type human IL-13 were used at concentrations of 50 ng/ml, 25 ng/ml and 25 ng/ml respectively.

Results

30 BIAcore binding assay data suggested that BAK278D6 but not BAK167A11 lineage had the required cross-reactivity profile for further therapeutic development (Table 2). This finding was supported by bioassay data demonstrating that BAK278D6 (Figure 4) and BAK502G9 (Figure 6) were able to neutralise

human IL-13, the human IL-13 (Q130R) variant and non-human primate IL-13 in the TF-1 cell proliferation assay with near equivalent potency. In contrast, although BAK615E3 (VH SEQ ID NO: 33; VL SEQ ID NO: 34) had a significantly increased 5 potency against human IL-13 over its parent BAK167A11 (VH SEQ ID NO: 23; VL SEQ ID NO: 24) in the TF-1 cell proliferation assay (Figure 1), neither clone bound non-human primate or variant IL-13 in the BIAcore binding assay.

10 *Germlining framework regions of BAK278D6 and BAK502G9*

The derived amino acid sequence of BAK278D6 VH (SEQ ID NO: 13) and VL (SEQ ID NO: 14) were aligned to the known human germline sequences in the VBASE database [73] and the closest germline identified by sequence similarity. The closest 15 germline for the VH domain of BAK278D6 (SEQ ID NO: 14) and its derivatives, was identified as DP14, a member of the VH1 family. The BAK278D6 VH has 9 changes from the DP14 germline within framework regions. The closest germline for the VL of BAK278D6 was identified as V_λ3 3h. The BAK278D6 VL domain (SEQ 20 ID NO: 14) has only 5 changes from the germline within framework regions. Framework regions of BAK278D6 and its derivatives were returned to germline by site directed mutagenesis (Stratagene Quikchange kit) to identically match native human antibodies.

25

EXAMPLE 5

Neutralisation potency of lead clones from targeted 30 optimisation of heavy chain CDR1 and heavy chain CDR2 sequences of BAK502G9 in the human IL-13 dependent TF-1 cell proliferation assay

A second phase of optimisation was performed using BAK502G9 sequence, with germlined framework regions, as a template. Selections were performed essentially as described in Example

1 on scFv repertoires in which either residues within the heavy chain CDR1 or heavy chain CDR2 of BAK502G9 had been randomised by mutagenesis. Unique clones from the selection output were identified by DNA sequencing and their

5 neutralising potency assessed as purified scFv preparations in the TF-1 cell proliferation assay as described in Example 2. Vectors were constructed for the most potent scFv clones to allow re-expression as whole human IgG4 antibody as described by Persic et al. (1997 Gene 187; 9-18) with a few

10 modifications. An oriP fragment was included in the vectors to facilitate use with HEK-EBNA 293 cells and to allow episomal replication. The VH variable domain was cloned into the polylinker between the secretion leader sequence and the human gamma 4 constant domain of the expression vector pEU8.1(+).

15 The VL variable domain was cloned into the polylinker between the secretion leader sequence and the human lambda constant domain of the expression vector pEU4.1(-).

Whole antibody was purified from conditioned media from EBNA-293 cells co-transfected with constructs expressing heavy and light chains by protein A affinity chromatography (Amersham Pharmacia). The purified antibody preparations were sterile filtered and stored at 4°C in phosphate buffered saline (PBS) prior to evaluation. Protein concentration was determined by

25 measuring absorbance at 280nm using the BCA method (Pierce). Reformatted human IgG4 whole antibodies were compared to commercially available anti-human IL-13 antibodies in the TF-1 proliferation assay described in Example 2.

30 Results

As demonstrated in Figure 5, the commercial antibody B-B13, (mouse IgG1 -Euroclone 5) was shown to be significantly more potent against human IL-13 than the commercial antibody JES10-5A2 (rat IgG1 - Biosource) with IC₅₀ of 1021pM and 471pM

respectively. Eight clones, namely, BAK1111D10, BAK1166G02, BAK1167F02, BAK1167F04, BAK1183H4, BAK1184C8, BAK1185E1, BAK1185F8, derived from BAK502G9 (and so "BAK502G9 lineage"), in which the heavy chain CDR1 or CDR2 had been targeted, 5 showed improved potency as scFv over the commercial antibodies. These improvements were maintained on conversion to whole antibody human IgG4. Each of these VH and VL domains individually and in the respective pairings of these claims represents an aspect or embodiment of the present invention, 10 as do specific binding members for IL-13 that comprise one or more of them, also specific binding members comprising one or more CDR's from the BAK502G9 lineage clones, preferably a VH domain comprising a BAK502G9 lineage set of HCDR's and/or a VL domain comprising a BAK502G9 lineage set of LCDR's. These may 15 be employed in any and all aspects of the invention as disclosed elsewhere herein. Derivatives of BAK502G9 as whole antibodies (IgG4) had an IC₅₀ ranging from 244pM to 283pM. BAK502G9 as a whole antibody IgG4 had an IC₅₀ of 384pM. In summary, major improvements in potency could be obtained by 20 targeting heavy chain CDR1 (SEQ ID NO:7) or CDR2 (SEQ ID NO: 8) of BAK502G9. Statistical comparisons to B-B13 were made using an ANOVA followed by a Dunnett's post test analysis (InStat software).

25 *Further characterisation*

Selected anti-human antibodies from the BAK278D6 lineage underwent further characterisation to determine their specificity. These included BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16) and its derivatives BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), which are representative examples of clones with modifications to heavy chain CDR1 and heavy chain CDR2 of BAK502G9 respectively.

EXAMPLE 6

Neutralisation potency of lead clones from targeted optimisation of heavy chain CDR1 and heavy chain CDR2 sequences of BAK502G9 against non-human primate IL-13 and an 5 IL-13 variant associated with asthma in the TF-1 factor dependent cell proliferation assay

Cross-reactivity of anti-human IL-13 antibodies was determined by their ability to inhibit non-human primate IL-13 and IL-13 10 variant mediated TF-1 cell proliferation as described in Example 4.

Results

Optimised anti-human IL-13 antibodies BAK1167F2 (VH SEQ ID NO: 15; VL SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38) maintained the specificity of their parent BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16) (Figure 6). Potency gains against wild type IL-13 were reflected in their ability to neutralise non-human primate IL-13 and an IL-13 variant with 20 equivalent potency. The IC₅₀ for BAK502G9 against human, human variant and non-human primate IL-13 were 1.4nM, 1.9nM and 2.0nM respectively. The IC₅₀ for BAK1167F2 against human, human variant and non-human primate IL-13 were 1.0nM, 1.1nM and 1.3nM respectively. The IC₅₀ for BAK1183H4 against human, human 25 variant and non-human primate IL-13 were 0.9nM, 1.0nM and 1.6nM respectively. These clones are suitable for therapeutic use.

EXAMPLE 7

30 Neutralising potency of lead anti-human IL-13 antibodies against native human IL-13 in HDEM-2 cell proliferation assay

The human IL-13 sequence has four potential N-glycosylation sites. The inventors have demonstrated the ability of BAK278D6

and its derivatives to neutralise recombinant IL-13 expressed either in bacterial or baculovirus expression systems. Although, there is evidence that many processing events known in mammalian systems do also occur in insects there are key 5 differences in protein glycosylation, particularly N-glycosylation [74].

The inventors investigated the ability of BAK278D6 derivatives to neutralise native IL-13 released from human cells.

10 HDLM-2 cells were isolated by Drexler et al [75] from a patient with Hodgkin's disease. Skinnider et al [76] demonstrated that HDLM-2 cell proliferation was in part dependent on autocrine and paracrine release of IL-13. Lead 15 anti-human IL-13 antibodies were assessed for their ability to inhibit HDLM-2 cell proliferation mediated by the release of native IL-13.

HDLM-2 cell assay protocol

20 HDLM-2 cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and maintained according to supplied protocols. Assay media comprised RPI-1640 with Glutamax I (Invitrogen) containing 20% foetal bovine serum. Prior to each assay, the cells were pelleted by 25 centrifugation at 300x g for 5 min, the media removed by aspiration and the cells resuspended in fresh media. This process was repeated three times and the cells were finally resuspended to a final concentration of 2×10^5 cells/ml in assay media. 50 μ l of resuspended cells were added to each 30 assay point in a 96 well assay plate. Test solutions of antibodies (in triplicate) were diluted to the desired concentration in assay media. An irrelevant isotype antibody not directed at IL-13 was used as a negative control. The appropriate test antibody in a total volume of 50 μ l / well

were added to the cells, each assay point giving a total assay volume of 100 μ l / well. Assay plates were incubated for 72 hours at 37°C under 5% CO₂. 25 μ l of tritiated thymidine (10 μ Ci/ml, NEN) was then added to each assay point and assay 5 plates were returned to the incubator for a further 4 hours. Cells were harvested on glass fibre filter plates (Perkin Elmer) using a cell harvester. Thymidine incorporation was determined using a Packard TopCount microplate liquid scintillation counter. Data were analysed using Graphpad Prism 10 software.

Results

As demonstrated in Figure 7, BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), and its derivatives BAK1183H4 (VH SEQ ID NO: 15; VL SEQ ID NO: 37) and BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) were able to cause a dose dependent inhibition of cell proliferation with relative potencies similar to those observed in other bioassays. IC₅₀ for BAK502G9, BAK1183H4, BAK1167F2 as human IgG4 were 4.6nM, 3.5nM and 1.1nM 20 respectively. IC₅₀ for the commercial antibodies JES10-5A2 and B-B13 were 10.7nM and 16.7nM respectively.

EXAMPLE 8

Neutralising potency of lead anti-human IL-13 antibodies 25 against IL-13 dependent responses in disease relevant primary cells

Secondary bioassays were performed using primary cells and readouts more relevant to airway disease. These included 30 eotaxin release from normal human lung fibroblasts (NHLF) and vascular adhesion molecule 1 (VCAM-1) upregulation on the surface of human umbilical vein endothelial cells (HUVEC). Both IL-13 dependent responses could contribute to eosinophil recruitment, a feature of the asthma phenotype [92].

NHLF assay protocol

IL-13 has been shown to cause eotaxin release from lung fibroblasts[77] [78] [79]. Factor dependent eotaxin release 5 from NHLF was determined by ELISA.

NHLF were obtained from Biowhittaker and maintained according to supplied protocols. Assay media was FGM-2 (Biowhittaker). Test solutions of antibody (in triplicate) were diluted to the 10 desired concentration in assay media. An irrelevant antibody not directed at IL-13 was used as a negative control. Recombinant bacterially-derived human IL-13 (Peprotech) was subsequently added to a final concentration of 10 ng/ml when mixed with the appropriate test antibody in a total volume of 15 200 µl. The concentration of IL-13 used in the assay was selected as the dose that gave an approximately 80% of the maximal response. All samples were incubated for 30 minutes at room temperature. Assay samples were then added to NHLF that had been preseeded at a density of 1×10^4 cells per well in 20 96-well assay plates. Assay plates were incubated at 37°C for 16-24 hours at 37°C under 5% CO₂. Assay plates were centrifuged at 300 x g for 5 minutes to pellet detached cells. Eotaxin 25 levels in the supernatant were determined by ELISA using reagents and methods described by the manufacturer (R&D Systems). Data were analysed using Graphpad Prism software.

Results

BAK278D6 lineage clones were able to inhibit human IL-13 dependent eotaxin release from NHLF. Relative potency was 30 similar to that observed in the TF-1 cell proliferation assay (Figure 8). BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) had IC₅₀ of 207pM, 118pM and 69pM respectively against 10 ng/ml human IL-13. Commercial

antibodies JES10-5A2 and B-B13 had IC₅₀ of 623pM and 219pM respectively.

HUVEC assay protocol

5 IL-13 has been shown to upregulate expression of VCAM-1 on cell surface of HUVECs [80, 81]. Factor dependent VCAM-1 expression was determined by detection of upregulation of VCAM-1 receptor cellular expression using a time-resolved fluorescence read out.

10

HUVEC were obtained from Biowhittaker and maintained according to supplied protocols. Assay media was EGM-2 (Biowhittaker). Test solutions of antibody (in triplicate) were diluted to the desired concentration in assay media. An irrelevant antibody 15 not directed at IL-13 was used as a negative control.

Recombinant bacterially derived human IL-13 (Peprotech) was added to a final concentration of 10 ng/ml when mixed with the appropriate test antibody in a total volume of 200 μ l. The concentration of IL-13 used in the assay was selected as the 20 dose that gave approximately 80% of the maximal response. All samples were incubated for 30 minutes at room temperature.

Assay samples were then added to HUVEC that had been preseeded at 4×10^4 cells per well in 96-well assay plates. Assay plates were incubated at 37°C for 16-20 hours under 5% CO₂.

25 Assay media was then removed by aspiration and replaced with blocking solution (PBS containing 4% dried Marvel® milk powder). Assay plates were incubated at room temperature for 1 hour at room temperature. Wells were washed three times with PBST Tween before 100 μ l (1:500 dilution in PBST/1% Marvel®) 30 of biotinylated anti-VCAM-1 antibody (Serotec) was added to each well. Assay plates were incubated at room temperature for 1 hour. Wells were washed three times with Delfia wash buffer (Perkin Elmer) before 100 μ l of Europium-labelled Streptavidin or anti-murine IgG1 (1:1000 dilution in Delfia assay buffer,

Perkin Elmer) was added to each well. Assay plates were then incubated at RT for 1 hour. Wells were washed 7 times with Delfia wash buffer (Perkin Elmer). Finally, 100 μ l of enhancement solution (Perkin Elmer) was added to each well and 5 fluorescence intensity was determined using the Wallac 1420 VICTOR2 plate reader (Standard Europium protocol). Data were analysed using Graphpad Prism software.

Results

10 Typical data for BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) as whole antibody human IgG4 are shown in Figure 9. Relative potency was similar to the observed in the TF-1 cell proliferation assay. IC₅₀ for 15 BAK502G9, BAK1183H4 and BAK1167F2 were 235pM, 58pM and 55pM respectively against 10ng/ml human IL-13.

EXAMPLE 9

20 Neutralisation potency of anti-IL-13 antibodies against IL-1 β and IL-4 dependent VCAM-1 upregulation

The specificity of the BAK278D6 lineage of clones was assessed in a modification of the HUVEC bioassay. Together with IL-13, both IL-4 and IL-1 β have been shown to upregulate expression 25 of VCAM-1 on cell surface of HUVECs [80, 81].

HUVEC assay protocol

The assay was performed essentially as described in Example 5 with the following modifications. Recombinant human IL-1 β and 30 IL-4 (R&D Systems) were used in place of human IL-13 at 0.5 ng/ml and 1 ng/ml respectively and represented the dose that gave approximately 80% of the maximal response.

Results

None of the clones evaluated from the BAK278D6 lineage neutralised VCAM-1 upregulation in response to either human IL-1 β or IL-4 and thus demonstrated specificity for IL-13
5 (Figure 10). IL-4 is most closely related to IL-13, sharing 30% sequence identity at the amino acid level [82].

EXAMPLE 10

10 *Neutralisation potency of BAK209B11 as a human IgG4 in a murine IL-13 dependent murine B9 cell proliferation assay*

BAK209B11, identified as an anti-murine IL-13 neutralising clone as a scFv as described in Example 1, was reformatted as a whole antibody human IgG4 as described in Example 5 and its 15 potency evaluated in the murine IL-13 dependent B9 cell proliferation assay. B9 is a murine B-cell hybridoma cell line [83]. B9 is factor dependent for survival and proliferation. In this respect B cells respond to murine IL-13 and are maintained in media containing human IL-6 (50pg/ml, R&D 20 Systems). Inhibition of murine IL-13 dependent proliferation was determined by measuring the reduction in incorporation of tritiated thymidine into the newly synthesized DNA of dividing cells.

25 *B9 cell assay protocol*

B9 cells were obtained from European Collection of Animal Cell Culture ECACC and maintained according to supplied protocols. The assay was performed essentially as described for the TF-1 assay in Example 2 but with the following modifications. Assay 30 media comprised RPMI-1640 with GLUTAMAX. I (Invitrogen) containing 5% foetal bovine serum (Hyclone) and 50 μ M 2-mercaptoethanol (Invitrogen). Recombinant bacterially derived murine IL-13 (Peprotech) replaced human IL-13 with a final assay concentration of 1ng/ml.

Results

BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) as a human IgG4 neutralised 1 ng/ml murine IL-13 with an IC₅₀ of 776pM in 5 the B9 assay (Figure 11). BAK209B11 therefore represents a useful tool to investigate the role of IL-13 in murine models of disease. This is clearly demonstrated in Example 12, which demonstrates the efficacy of BAK209B11 in a murine model of acute pulmonary inflammation.

10

EXAMPLE 11

Affinity determination of anti-IL-13 antibodies by BIAcore analysis

15 The affinity of BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38) for human IL-13 and BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) for murine IL-13 as human IgG4 were determined by surface plasmon resonance 20 measurements using a BIAcore 2000 Biosensor (BIAcore AB) essentially as described in [72]. In brief, antibodies were coupled to CM5 sensorchips using an amine coupling kit (BIAcore) at a surface density of approximately 500Ru and a serial dilution of IL-13 (between 50nM to 0.78nM) in HBS-EP 25 buffer was passed over the sensorchip surface. The resulting sensorgrams were evaluated using BIA evaluation 3.1 software to provide kinetic data.

Results

30 BAK502G9, BAK1167F2 and BAK1183H4 IgG4 bound human IL-13 with high affinity with Kd of 165pM, 136pM and 81pM respectively corresponding to their relative potency in cell based assays. BAK209B11 bound murine IL-13 with affinity of 5.1nM (Table 3).

EXAMPLE 12***Efficacy of BAK209B11 in a murine model of acute allergic pulmonary inflammation***

5

Murine model of acute allergic pulmonary inflammation

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti-murine IL-13 neutralising human IgG4 antibody, was investigated in a murine of acute allergic pulmonary

10 inflammation. This model was performed essentially as described by Riffo-Vasquez et al [84] and is characterised at its endpoint by increased bronchial alveolar lavage (BAL) IL-13 (Figure 12), cellular infiltration into the lung and BAL (Figure 13), increased serum IgE levels and airways

15 hyperresponsiveness (AHR).

Model protocol

Female Balb/C mice (Charles River UK) were treated with either anti-murine IL-13 antibody BAK209B11 (at 12, 36, 119 or 357 µg 20 doses) or an isotype matched control antibody (357 µg dose).

On days 0 and 7, mice in each group were sensitised by intraperitoneal injection of 10µg of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2% Al₂O₃ (Rehydragel) as an adjuvant). A separate control group of non-sensitised mice 25 received an equal volume of the vehicle. Mice were challenged with ovalbumin on days 14, 15 and 16. Ovalbumin was diluted to 1% (w/v) in sterile saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss 30 Ultraneb 2000 nebuliser (Sunrise Medical) in a series of three exposures of 20 minutes separated by 1 hour intervals.

BAK209B11 or an irrelevant human IgG4 were administered intravenously, 1 day prior to first challenge and then 2 hours

prior to each subsequent challenge (4 doses in total). The model ended at day 17, 24 hours post final challenge. Blood (serum) and BAL were collected. Serum was assayed for total IgE. BAL was obtained by injecting 3 aliquots of saline 5 (0.3ml, 0.3ml and 0.4ml) and pooling samples. Total leukocytes and differential cell counts were obtained from BAL cells.

Results

Ovalbumin challenge of sensitised mice caused a significant 10 (p<0.05) increase in total BAL cell recruitment over non-sensitised but challenged animals. This recruitment was dose-dependently inhibited by BAK209B11; significant (p<0.05) inhibition was seen with $\geq 36\mu\text{g}$ BAK209B11, but not control antibody (Figure 13). Similar effects were also seen on 15 eosinophils (Figure 14) and neutrophils (Figure 15) with significant (p<0.05) inhibition of cellular influx at a minimum BAK209B11 dose of $36\mu\text{g}$. This inhibition was not seen with the control antibody. Lymphocytes were also induced in sensitised but not non-sensitised mice upon challenge. This 20 induction was dose-dependently inhibited by BAK209B11, with maximal inhibition seen with $36\mu\text{g}$ BAK209B11. Control antibody had no effect (Figure 16). Although monocyte/macrophages were not induced in sensitised animals when compared to non-sensitised animals, background levels were depressed by $\geq 36\mu\text{g}$ 25 BAK209B11, but not by control antibody (Figure 17). Serum IgE levels were significantly increased in sensitised animals when compared to non-sensitised after challenge (p<0.05). This increase was decreased after treatment with $36\mu\text{g}$ BAK209B11 but not by the control antibody.

30 In summary, systemic administration of BAK209B11, a murine IL-13 neutralising antibody, but not control antibody inhibited inflammatory cell influx and the upregulation of serum IgE

levels caused by sensitisation and subsequent challenge with ovalbumin in a murine model of allergic inflammation.

Examples 13 to 20 are prophetic.

5

EXAMPLE 13

Efficacy of BAK209B11 in the Lloyd murine model of acute pulmonary inflammation

10 *Murine model of acute allergic pulmonary inflammation*

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti murine IL-13 neutralising antibody, was investigated in a second murine model of acute allergic pulmonary inflammation. This model was performed essentially as 15 described by McMillan et al. [85] and is characterised at its endpoint by increased BAL and lung tissue IL-13, cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

20 *Model protocol*

Female Balb/C mice (Charles River UK) were administered with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody, as follows. On days 0 and 12, mice in each group were sensitised (SN) by intraperitoneal 25 injection of 10 μ g of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2mg Al(OH)₃ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 19, 30, 20, 21, 22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical). On days 18, 19, 20, 21, 22, 23 and 24 mice were

administered with various intraperitoneal doses (237 μ g, 23.7 μ g or 2.37 μ g; denoted in figure 21 by H, M and L) of anti-murine IL-13 antibody BAK209B11 muIgG1 or an isotype matched control antibody (237 μ g). Airway function was assessed on days 0 and 5 25 by increasing methacholine challenges and monitored using conscious plethysmography (Buxco). PC₅₀ (concentration of methacholine required to increase baseline PenH by 50%) was estimated for individual mice at both day 0 and day 25 from 4 parameter unfixed curve fitting of methacholine dose-response curves.

10 The model ended at day 25, 24 hours post final challenge. Blood, serum, BAL and lung tissue were collected.

15 **Results**

Lung function was evaluated for individual animals at day 0 (pre-treatment) and at day 25 (post-challenge) and was quantitated by calculating PC₅₀ values (concentration of methacholine required to increase baseline PenH by 50%) (Figure 21A). An individuals airways hyperresponsiveness (AHR) was determined by the change in log PC₅₀ at day 25 versus day 0 (log day 25 PC₅₀ - log day 0 PC₅₀). This delta logPC₅₀ was the primary endpoint of the study; PC₅₀ data log-transformed because of requirements of endpoint ANOVA. 25 Individual changes were averaged within groups to generate group average delta log PC₅₀ (as shown in Figure 21B).

Ovalbumin challenge of sensitised mice caused a significant AHR compared to non-sensitised and challenged mice ($p<0.01$). 30 BAK209B11 caused a clear and dose-dependent decrease in AHR whereas the control antibody had no effect.

EXAMPLE 14***Efficacy of BAK209B11 in the Gerard murine model of acute pulmonary inflammation***

5

Murine model of acute allergic pulmonary inflammation

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti-murine IL-13 neutralising human IgG4 antibody, was investigated in a third murine model of acute allergic pulmonary inflammation. This model was performed essentially as described by Humbles et al. [86] and is characterised at its endpoint by increased BAL and lung tissue IL-13, cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

15

Model protocol

Female Balb/C mice (Charles River UK) were administered with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody. On days 0, 7 and 14, mice in each group were sensitised (SN) by intraperitoneal injection of 10 μ g of ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 1.125mg Al(OH)₃ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 21, 22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical).

The model ended at day 25, 24 hours post challenge. Blood, serum, BAL and lung tissue were collected.

EXAMPLE 15***Efficacy of BAK209B11 in the Lloyd chronic model of pulmonary inflammation*****5 *Murine model of chronic allergic pulmonary inflammation***

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti murine IL-13 neutralising human IgG4 antibody, was investigated in a model of chronic allergic pulmonary inflammation. This model was performed essentially as

10 described by Temelkovski et al. [87] and is characterised at its endpoint by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

15 *Model protocol*

Female Balb/C mice (Charles River UK) were dosed with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody. On days 0 and 11, mice in each group were sensitised (SN) by intraperitoneal injection of 10 μ g of 20 ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2mg Al(OH)₃ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 18, 19, 20, 21, 22, 23, 25 28, 30, 32, 35, 37, 39, 42, 44, 46, 49 and 51. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical).

30

The model ended at day 52, 24 hours post challenge. Blood, serum, BAL and lung tissue were collected.

EXAMPLE 16

Efficacy of anti-human IL-13 antibodies against exogenous human IL-13 administered to the murine air pouch model

The effect of anti-human IL-13 antibodies on the pro-inflammatory action of human IL-13 was investigated in a basic murine model. This model was performed essentially as described by Edwards et al [93] and was characterised at its endpoint by cellular infiltration into the airpouch.

10 *Model protocol*

An air pouch was created on the back of female Balb/C mice by subcutaneous injection of 2.5mL of sterile air at day 0. The air pouch was reinflated with another 2.5mL sterile air at day 3. 2 μ g huIL-13 in 0.75% CMC was injected directly into the pouch at day 6. 24 hours later the mice were killed and the air pouch lavaged with 1mL heparinised saline. Antibody treatments were either given with the huIL-13 (into the pouch) or given systemically.

20 *Results*

Human IL-13, injected into the airpouch (i.po.), caused a significantly increased infiltration of total leukocytes (p<0.01) and eosinophils (p<0.01) at 24 hours post-challenge versus vehicle (0.75% carboxymethyl cellulose (CMC) in saline i.po.) treated mice.

Locally administered BAK502G9 (200mg, 20mg or 2mg intrapouch) significantly and dose-dependently inhibited the total leukocyte (p<0.01) and eosinophil (p<0.01) infiltration into the air pouch caused by 2 μ g huIL-13 in 0.75% CMC.

Systemically administered BAK209B11 (30mg/kg, 10mg/kg and 1mg/kg) also significantly and dose-dependently inhibited the

total leukocyte ($p<0.01$) and eosinophil ($p<0.01$) infiltration into the air pouch caused by $2\mu\text{g}$ huIL-13 in 0.75% CMC.

EXAMPLE 17

5 *Generation of human IL-13 knock-in / murine IL-13 knock out transgenic mice for the purposes of evaluating the efficacy of anti-human IL-13 antibodies in models of pulmonary allergic inflammation*

10 The present inventors have generated mice which express human, rather than murine IL-13 by gene targeting. The mouse IL-13 gene has been replaced from start to stop codon with the relevant portion of the human IL-13 gene. This mouse strain expresses human IL-13, rather than mouse IL-13, in response to

15 the same stimuli as in the wild-type mouse, as the endogenous IL-13 promoter and IL-13 pA tail remaining unchanged. It has been shown that human IL-13 can bind to and signal through mouse IL-13 receptors to generate the same physiological consequences as signalling caused by mouse IL-13 ligating

20 mouse IL-13 receptors. For example exogenous human IL-13 caused inflammatory cell recruitment into the murine air pouch (Figure 18). These transgenic animals allow us to evaluate non-murine cross reactive anti-human IL-13 antibodies in established murine models of disease.

25 This mouse has been used in the acute allergic airway inflammation models (as described in examples 18 and 19) and chronic allergic airway inflammation models (as described in Example 20) allowing the evaluation of anti-human IL-13 antibody pharmacology in allergic airway disease.

EXAMPLE 18

Efficacy of anti-human IL-13 antibodies in the huIL-13-transgenic Lloyd murine model of acute pulmonary inflammation

Murine model of acute allergic pulmonary inflammation

The effect of anti human IL-13 neutralising human IgG4 antibodies were investigated in a murine model of acute 5 allergic pulmonary inflammation using the transgenic mice generated in example 17. This model was performed essentially as described by McMillan et al. [85] and example 13. The model was characterised at its endpoint by increased BAL and lung tissue IL-13, cellular infiltration into the lung and 10 BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

Model protocol

The protocol for this model was as described in Example 13 15 except that anti-human IL-13 antibodies were dosed instead of BAK209B11.

EXAMPLE 19

Efficacy of anti-human IL-13 antibodies in the huIL-13- 20 transgenic Gerard murine model of acute pulmonary inflammation

Murine model of acute allergic pulmonary inflammation

The effect of anti human IL-13 neutralising human IgG4 25 antibodies were investigated in another murine model of acute allergic pulmonary inflammation using the transgenic mice generated in example 17. This model was performed essentially as described by Humbles et al, [86] and in example 14. The model is characterised at its endpoint by increased BAL and 30 lung tissue IL-13, cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

Model protocol

The protocol for this model was as described in Example 14 except that anti-human IL-13 antibodies were dosed instead of BAK209B11.

5

EXAMPLE 20

Efficacy of anti-human IL-13 antibodies in the huIL-13-transgenic Lloyd chronic model of pulmonary inflammation

10 The effect of anti human IL-13 neutralising human IgG4 antibodies were investigated in a model of chronic allergic pulmonary inflammation using the transgenic mice generated in example 17. This model was performed essentially as described by Temelkovski et al. [87] and in Example 15 and is

15 characterised at its endpoint by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

Model protocol

20 The protocol for this model was as described in Example 15 except that anti-human IL-13 antibodies were dosed instead of BAK209B11

EXAMPLE 21

25 *Pharmacokinetics and pharmacodynamics of anti-human IL-13 antibodies in Ascaris.suum-allergic cynomolgus monkeys*

The pharmacokinetics and pharmacodynamics of 502G9 were evaluated in 4 allergic but non-challenged cynomolgus primates 30 (2 male/2 female) after a single 10mg/kg i.v bolus dose. The experiment ran for 29 days. The antibody's pharmacokinetic parameters were determined from a geomean average serum-drug concentration curve and are detailed below in Table 4.

In the same study serum IgE concentrations were also followed using a human IgE ELISA kit (Bethyl laboratories, USA).

Results

5 Serum IgE concentrations were significantly reduced after a single 10mg/kg i.v bolus dose of BAK502G9, from 100 % control levels (predose) to 66 ± 10% of control values (p<0.05), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovered to 88 ± 8 % of control levels by day 10 22 (see Figure 20). Again these data were derived by normalising each animals serum IgE concentration to predose levels, where predose concentrations was 100%, and then averaging the curves from the 4 animals tested.

15 **EXAMPLE 22**

Efficacy of anti-human IL-13 antibodies in cynomolgus models of dermal allergy

20 The effects of anti-human IL-13 neutralising human IgG4 antibodies were investigated in a primate model of acute allergic dermal inflammation. This model was performed by injecting human IL-13 and *A.suum* antigen intradermally into cynomolgus monkeys. 24-96h later, dermal biopsies and serum samples were taken. The model was characterised at its 25 endpoint by cellular infiltration into the skin.

EXAMPLE 23

Efficacy of anti-human IL-13 antibodies in cynomolgus models of pulmonary allergy

30 The effect of anti human IL-13 neutralising human IgG4 antibodies were investigated in a primate model of acute allergic pulmonary inflammation. This model was performed by exposing *a.suum*-allergic cynomolgus primates to nebulised

a.suum antigen, thereby generating an allergic reaction. This allergy was characterized at its end point by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness.

5

Pharmacodynamics were additionally evaluated *ex vivo* using a flow cytometric method. CD23 is the high affinity IgE receptor and can be expressed on peripheral human blood mononuclear cells. CD23 expression can be induced, in terms of the number of cells expressing CD23 and also in how much CD23 each cell expresses by both IL-13 and IL-4. The IL-13, but not IL-4, mediated response can be inhibited by anti-human IL-13 antibodies.

15 Blood, serum, BAL and lung tissue were collected. Serum IgE levels were monitored by ELISA. Serum from BAK502G9 treated cynomolgus monkeys was shown to inhibit the expression of CD23 on human peripheral blood mononuclear cells induced by IL-13 but not IL-4. The magnitude of this inhibition was consistent 20 with the serum BAK502G9 levels predicted by PK ELISA.

REFERENCES

1. McKenzie, A.N., et al. *J Immunol*, 1993. **150**(12): p. 5436-44.
- 25 2. Minty, A., et al. *Nature*, 1993. **362**(6417): p. 248-50.
3. Nakamura, Y., et al. *Am J Respir Cell Mol Biol*, 1996. **15**(5): p. 680-7.
4. Robinson, D.S., et al. *N Engl J Med*, 1992. **326**(5): p. 298-304.
- 30 5. Walker, C., et al. *Am J Respir Crit Care Med*, 1994. **150**(4): p. 1038-48.
6. Humbert, M., et al. *Am J Respir Crit Care Med*, 1996. **154**(5): p. 1497-504.
7. Corrigan, C.J. and A.B. Kay *Int Arch Allergy Appl*

Immunol, 1991. 94(1-4): p. 270-1.

8. Bentley, A.M., et al. Am J Respir Cell Mol Biol, 1993. 8(1): p. 35-42.

9. Murata, T., et al. Int J Hematol, 1999. 69(1): p. 13-20.

5 10. Andrews, A.L., et al. J Biol Chem, 2002. 277(48): p. 46073-8.

11. Miloux, B., et al. FEBS Lett, 1997. 401(2-3): p. 163-6.

12. Hilton, D.J., et al. Proc Natl Acad Sci U S A, 1996. 93(1): p. 497-501.

10 13. Kuperman, D., et al. J Exp Med, 1998. 187(6): p. 939-48.

14. Nelms, K., et al. Annu Rev Immunol, 1999. 17: p. 701-38.

15. Zhang, J.G., et al. J Biol Chem, 1997. 272(14): p. 9474-80.

16. Caput, D., et al. J Biol Chem, 1996. 271(28): p. 16921-6.

15 17. Kawakami, K., et al. Blood, 2001. 97(9): p. 2673-9.

18. Wood, N., et al. J Exp Med, 2003. 197(6): p. 703-709.

19. Chiaramonte, M.G., et al. J Exp Med, 2003. 197(6): p. 687-701.

20. Beasley, R., et al. J Allergy Clin Immunol, 2000. 105(2 Pt 2): p. S466-72.

21. Peat, J.K. and J. Li J Allergy Clin Immunol, 1999. 103(1 Pt 1): p. 1-10.

22. Society, B.T., *British guideline on the management of asthma*. Thorax, 2003. 58 Suppl 1: p. i1-94.

25 23. GINA, *Global Strategy for Asthma Management and Prevention*. 2002, National Institute of Health.

24. Milgrom, H., B. Bender, and F. Wamboldt. Ann Allergy Asthma Immunol, 2002. 88(5): p. 429-31.

25. Fish, L. and C.L. Lung, *Adherence to asthma therapy*. Ann Allergy Asthma Immunol, 2001. 86(6 Suppl 1): p. 24-30.

30 26. Bender, B.G. J Allergy Clin Immunol, 2002. 109(6 Suppl): p. S554-9.

27. Wills-Karp, M., et al. Science, 1998. 282(5397): p. 2258-61.

28. Grunig, G., et al. *Science*, 1998. **282**(5397): p. 2261-3.

29. Venkayya, R., et al. *Am J Respir Cell Mol Biol*, 2002. **26**(2): p. 202-8.

5 30. Morse, B., et al. *Am J Physiol Lung Cell Mol Physiol*, 2002. **282**(1): p. L44-9.

31. Zhu, Z., et al. *J Clin Invest*, 1999. **103**(6): p. 779-88.

32. Walter, D.M., et al. *J Immunol*, 2001. **167**(8): p. 4668-75.

33. Cohn, L., J.S. Tepper, and K. Bottomly. *J Immunol*, 1998. **161**(8): p. 3813-6.

10 34. Taube, C., et al. *J Immunol*, 2002. **169**(11): p. 6482-9.

35. Yang, E.S., et al. *J. Allergy Immunol.*, 2002. **109**: p. A168.

36. Bleasdale, K., et al. *J Immunol*, 2001. **166**(8): p. 5219-24.

15 37. Heinzmann, A., et al. *Hum Mol Genet*, 2000. **9**(4): p. 549-59.

38. Howard, T.D., et al. *Am J Hum Genet*, 2002. **70**(1): p. 230-6.

39. Kauppi, P., et al. *Genomics*, 2001. **77**(1-2): p. 35-42.

40. Graves, P.E., et al. *J Allergy Clin Immunol*, 2000. **105**(3): p. 506-13.

20 41. Arima, K., et al. *J Allergy Clin Immunol*, 2002. **109**(6): p. 980-7.

42. van der Pouw Kraan, T.C., et al. *Genes Immun*, 1999. **1**(1): p. 61-5.

25 43. Humbert, M., et al. *J Allergy Clin Immunol*, 1997. **99**(5): p. 657-65.

44. Kotsimbos, T.C., P. Ernst, and Q.A. Hamid, *Proc Assoc Am Physicians*, 1996. **108**(5): p. 368-73.

45. Komai-Koma, M., F.Y. Liew, and P.C. Wilkinson, *J Immunol*, 30 1995. **155**(3): p. 1110-6.

46. Naseer, T., et al. *Am J Respir Crit Care Med*, 1997. **155**(3): p. 845-51.

47. Huang, S.K., et al. *J Immunol*, 1995. **155**(5): p. 2688-94.

48. Kroegel, C., et al. *Eur Respir J*, 1996. **9**(5): p. 899-904.

49. Ohshima, Y., et al. *Pediatr Res*, 2002. **51**(2): p. 195-200.

50. Hasegawa, M., et al. *J Rheumatol*, 1997. **24**(2): p. 328-32.

51. Hancock, A., et al. *Am J Respir Cell Mol Biol*, 1998. **18**(1): p. 60-5.

52. Lee, C.G., et al. *J Exp Med*, 2001. **194**(6): p. 809-21.

53. Jain-Vora, S., et al. *Am J Respir Cell Mol Biol*, 1997. **17**(5): p. 541-51.

54. Fallon, P.G., et al. *J Immunol*, 2000. **164**(5): p. 2585-91.

55. Chiaramonte, M.G., et al. *J Clin Invest*, 1999. **104**(6): p. 777-85.

56. Chiaramonte, M.G., et al. *Hepatology*, 2001. **34**(2): p. 273-82.

57. Sluiter, H.J., et al. *Eur Respir J*, 1991. **4**(4): p. 479-89.

58. Zheng, T., et al. *J Clin Invest*, 2000. **106**(9): p. 1081-93.

59. Tashkin, D.P., et al., *Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group*. *Am J Respir Crit Care Med*, 1996. **153**(6 Pt 1): p. 1802-11.

60. Van Der Pouw Kraan, T.C., et al. *Genes Immun*, 2002. **3**(7): p. 436-9.

61. Skinnider, B.F., et al. *Blood*, 2001. **97**(1): p. 250-5.

62. Kapp, U., et al. *J Exp Med*, 1999. **189**(12): p. 1939-46.

63. Fiumara, P., F. Cabanillas, and A. Younes, *Blood*, 2001. **98**(9): p. 2877-8.

64. Terabe, M., et al. *Nat Immunol*, 2000. **1**(6): p. 515-20.

65. Ahlers, J.D., et al. *Proc Natl Acad Sci U S A*, 2002. **99**(20): p. 13020-5.

66. Hutchings, C., *Generation of Naïve Human Antibody Libraries, in Antibody Engineering*, R. Kontermann and S. Dubel, Editors. 2001, Springer Laboratory Manuals, Berlin. p. 93-108.

67. Vaughan, T.J., et al. *Nat Biotechnol*, 1996. **14**(3): p. 309-14.

68. Kitamura, T., et al. *Blood*, 1989. **73**(2): p. 375-80.

69. Lefort, S., et al. *FEBS Lett*, 1995. **366**(2-3): p. 122-6.

5 70. Osbourn, J.K., et al. *Immunotechnology*, 1996. **2**(3): p. 181-96.

71. Howard, T.D., et al. *Am J Respir Cell Mol Biol*, 2001. **25**(3): p. 377-84.

10 72. Karlsson, R., A. Michaelsson, and L. Mattsson, *J Immunol Methods*, 1991. **145**(1-2): p. 229-40.

73. Tomlinson, VBASE. 1997, MRC Centre for Protein Engineering, Cambridge, UK.

74. Altmann, F., et al. *Glycoconj J*, 1999. **16**(2): p. 109-23.

75. Drexler, H.G., et al. *Leuk Res*, 1986. **10**(5): p. 487-500.

15 76. Skinnider, B.F., U. Kapp, and T.W. Mak, *Leuk Lymphoma*, 2002. **43**(6): p. 1203-10.

77. Terada, N., et al. *Clin Exp Allergy*, 2000. **30**(3): p. 348-55.

78. Wenzel, S.E., et al. *J Immunol*, 2002. **169**(8): p. 4613-9.

20 79. Richter, A., et al. *Am J Respir Cell Mol Biol*, 2001. **25**(3): p. 385-91.

80. Bochner, B.S., et al. *J Immunol*, 1995. **154**(2): p. 799-803.

81. Kotowicz, K., et al. *Int Immunol*, 1996. **8**(12): p. 1915-25.

25 82. McKenzie, A.N., et al. *Journal of Immunology*, 1993. **150**(12): p. 5436-44.

83. Bouteiller, C.L., et al. *J Immunol Methods*, 1995. **181**(1): p. 29-36.

30 84. Riffo-Vasquez, Y., et al. *Clin Exp Allergy*, 2000. **30**(5): p. 728-38.

85. McMillan, S.J., et al. *J Exp Med*, 2002. **195**(1): p. 51-7.

86. Humbles, A.A., et al. *Proc Natl Acad Sci U.S.A*, 2002. **99**(3): p. 1479-84.

87. Temelkovski, J., et al. Thorax, 1998. 53(10): p. 849-56.

88. Belvisi, M.G., et al., Pulm Pharmacol Ther, 2001. 14(3): p. 221-7.

5

89. Barnes, P.J., et al. Eur Respir J, 1996. 9(4): p. 636-42.

90. Barnes, P.J., Pharmacol Ther, 2003. 97(1): p. 87-94.

10 91. Wardlaw, A.J., Clin Med, 2001. 1(3): p. 214-8.

92. Edwards, J.C., et al. J Pathol, 1981. 134(2): p. 147-56.

15 93. McDonough, J.E., et al. W.M. Elliot, and J.C. Hogg. *TGF- β Isoform and IL-13 Immunostaining on Lung Tissue from Patients with COPD*. in ATS 99th International Conference. 2003. Seattle.

94. Wold, et al. Multivariate data analysis in chemistry. Chemometrics - Mathematics and Statistics in Chemistry (Ed.: B. Kowalski), D. Reidel Publishing Company, Dordrecht, Holland, 1984 (ISBN 90-277-1846-6).

20 95. Norman et al. Applied Regression Analysis. Wiley-Interscience; 3rd edition (April 1998) ISBN: 0471170828

25 96. Abraham Kandel, Eric Backer. Computer-Assisted Reasoning in Cluster Analysis. Prentice Hall PTR; (May 11, 1995), ISBN: 0133418847

30 97. Wojtek Krzanowski. Principles of Multivariate Analysis: A User's Perspective (Oxford Statistical Science Series, No 22 (Paper)). Oxford University Press; (December 2000), ISBN: 0198507089

98. Ian H. Witten, Eibe Frank. *Data Mining: Practical Machine Learning Tools and Techniques with Java Implementations*. Morgan Kaufmann; (October 11, 1999), ISBN: 1558605525

5

99. David G. T. Denison (Editor), Christopher C. Holmes, Bani K. Mallick, Adrian F. M. Smith. *Bayesian Methods for Nonlinear Classification and Regression (Wiley Series in Probability and Statistics)*. John Wiley & Sons; (July 10 2002), ISBN: 0471490369

100. Arup K. Ghose, Vellarkad N. Viswanadhan. *Combinatorial Library Design and Evaluation Principles, Software, Tools, and Applications in Drug Discovery*. ISBN: 0-8247-15 0487-8

101. Chothia C. et al. *Journal Molecular Biology* (1992) 227, 799-817.

20 102. Al-Lazikani, et al. *Journal Molecular Biology* (1997) 273(4), 927-948.

103. Chothia, et al. *Science*, 233, 755-758 (1986).

25 104. Whitelegg, N.R.J. and Rees, A.R (2000). *Prot. Eng.*, 13, 819-824.

105. Available from Accelrys Inc.

30 [106] Guex, N. and Peitsch, M.C. (1997). *Electrophoresis* (1997) 18, 2714-2723.

107. Kabat E A et al (1991): Sequences of Proteins of

Immunological Interest, 5th Edition. US Department of Health and Human Services, Public Service, NIH, Washington.

5 108. Kontermann R and Dubel Stefan; (2001) Antibody Engineering, Springer Laboratory Manuals.

109. Mendez et al (1997); Nature Genetics Vol. 2: 146-156.

10 110. Csonka E et al (2000) Journal of Cell Science, 113: 3207-3216.

111. Vanderbyl S et al (2002) Molecular Therapy, 5(5): 10.

15 112. Marasco WA (1997) Gene Therapy, 4(1): 11.

113. Hanes J et al (2000). Methods in Enzymology, Vol 328:24.

114. Li et al (2003). Abstract for poster [605] submitted at
20 The American Thoracis Society Annual Meeting, 2003,
Seattle.

115. Koide et al (1998). Journal of Molecular Biology, Vol
284:1141-1151.

25 116. Nygren et al (1997). Current Opinion in Structural Biology, Vol 7:463-469.

117. Heller, F., et al. (2002) Immunity, 17(5):629-38.

30 118. Inoue, S., et al. (1999) Am J Gastroenterol, 94(9):2441-6.

Table 1

Koban numbering	Koban numbering	LCDR1		LCDR2		LCDR3	
		Hard	Soft	Hard	Soft	Hard	Soft
BAN272806	N Y Q L S W I S A N N G D T H Y G Q E F Q G D S S N W A R W Y P D L G G H N I G S K L V H D D D D R P S O V N D T O S D P P T						
BAK502659	PD	D D D S	T I				
BAK110784	PD	Q T	V	G L	E L		
BAK110762	PD	Q T	V	N D A T	Q		
BAK110543	PD			A T P D	Q		
BAK110561	PD			R P T D	I N		
BAK111019	PD				N	S	
BAK110455	PD					S	
BAK110862	PD	T I		G B	V S		
BAK110458	PD			R H I D	V I		
BAK110556	PD	D		R A D	H H		
BAK110774	PD	D T	V	D D R T	T Q		
BAK110965	PD			H Y D G	N Q		
BAK110334	PD			R S D	Q I		
BAK110302	PD			C I D	V L		
BAK110459	PD			R A D	H H		
BAK110508	PD					S	
BAK110508	PD	L T	V	G H	L		
BAK110355	PD			G P S K	S S		
BAK110570	PD			R P R D	T H		
BAK110504	PD			G R S	A L		
BAK110305	PD					S	
BAK110107	PD	Q T	V				
BAK110504	PD	B T	I				
BAK110107	PD	D T	I				
BAK110509	PD	Q T	I				
BAK110305	PD						
BAK110107	PD	Q T	V				
BAK110504	PD	B T	I				
BAK110509	PD	Q T	I				
BAK110505	PD						
BAK110505	PD						
ALA V126L	O						
ALA V126M	O						
ALA V126C	O						
ALA V126V	O						
ALA V126K	O						
ALA V126Y	O						
ALA V126F	O						
ALA V126R	O						
ALA V126T	O						
BAK100101C10	RD						
BAK101097	RD						
BAK100083	RD						
BAK100083	RD						
BAK100084	RD						
BAK100102	RD						
BAK100779	RD						
BAK10101019	RD						
BAK100102	RD						
ALA V126A	O						
BAK100102	RD						
BAK100704	RD						
ALA V126H	O						
BAK105453	RD						
BAK105067	RD	D					
BAK101059	RD						
ALA V126S	O						
BAK105002	RD						
BAK105010	RD						
BAK105046	RD						
BAK102105	RD						
BAK105010	RD						
ALA V126	O						
BAK105055	RD						
BAK104955	RD						
BAK104955	RD						

Table 1 (cont'd)

Kabat numbering	MC01R		MC02R		MC03R		LC01R		LC02R		LC03R		LC04R		LC05R		LC06R		LC07R		LC08R		LC09R		LC10R		LC11R		LC12R		LC13R		LC14R		LC15R		LC16R		LC17R		LC18R		LC19R		LC20R		LC21R		LC22R		LC23R		LC24R		LC25R		LC26R		LC27R		LC28R		LC29R		LC30R		LC31R		LC32R		LC33R		LC34R		LC35R		LC36R		LC37R		LC38R		LC39R		LC40R		LC41R		LC42R		LC43R		LC44R		LC45R		LC46R		LC47R		LC48R		LC49R		LC50R		LC51R		LC52R		LC53R		LC54R		LC55R		LC56R		LC57R		LC58R		LC59R		LC60R		LC61R		LC62R		LC63R		LC64R		LC65R		LC66R		LC67R		LC68R		LC69R		LC70R		LC71R		LC72R		LC73R		LC74R		LC75R		LC76R		LC77R		LC78R		LC79R		LC80R		LC81R		LC82R		LC83R		LC84R		LC85R		LC86R		LC87R		LC88R		LC89R		LC90R		LC91R		LC92R		LC93R		LC94R		LC95R		LC96R		LC97R		LC98R		LC99R		LC100R		LC101R		LC102R		LC103R		LC104R		LC105R		LC106R		LC107R		LC108R		LC109R		LC110R		LC111R		LC112R		LC113R		LC114R		LC115R		LC116R		LC117R		LC118R		LC119R		LC120R		LC121R		LC122R		LC123R		LC124R		LC125R		LC126R		LC127R		LC128R		LC129R		LC130R		LC131R		LC132R		LC133R		LC134R		LC135R		LC136R		LC137R		LC138R		LC139R		LC140R		LC141R		LC142R		LC143R		LC144R		LC145R		LC146R		LC147R		LC148R		LC149R		LC150R		LC151R		LC152R		LC153R		LC154R		LC155R		LC156R		LC157R		LC158R		LC159R		LC160R		LC161R		LC162R		LC163R		LC164R		LC165R		LC166R		LC167R		LC168R		LC169R		LC170R		LC171R		LC172R		LC173R		LC174R		LC175R		LC176R		LC177R		LC178R		LC179R		LC180R		LC181R		LC182R		LC183R		LC184R		LC185R		LC186R		LC187R		LC188R		LC189R		LC190R		LC191R		LC192R		LC193R		LC194R		LC195R		LC196R		LC197R		LC198R		LC199R		LC200R		LC201R		LC202R		LC203R		LC204R		LC205R		LC206R		LC207R		LC208R		LC209R		LC210R		LC211R		LC212R		LC213R		LC214R		LC215R		LC216R		LC217R		LC218R		LC219R		LC220R		LC221R		LC222R		LC223R		LC224R		LC225R		LC226R		LC227R		LC228R		LC229R		LC230R		LC231R		LC232R		LC233R		LC234R		LC235R		LC236R		LC237R		LC238R		LC239R		LC240R		LC241R		LC242R		LC243R		LC244R		LC245R		LC246R		LC247R		LC248R		LC249R		LC250R		LC251R		LC252R		LC253R		LC254R		LC255R		LC256R		LC257R		LC258R		LC259R		LC260R		LC261R		LC262R		LC263R		LC264R		LC265R		LC266R		LC267R		LC268R		LC269R		LC270R		LC271R		LC272R		LC273R		LC274R		LC275R		LC276R		LC277R		LC278R		LC279R		LC280R		LC281R		LC282R		LC283R		LC284R		LC285R		LC286R		LC287R		LC288R		LC289R		LC290R		LC291R		LC292R		LC293R		LC294R		LC295R		LC296R		LC297R		LC298R		LC299R		LC300R		LC301R		LC302R		LC303R		LC304R		LC305R		LC306R		LC307R		LC308R		LC309R		LC310R		LC311R		LC312R		LC313R		LC314R		LC315R		LC316R		LC317R		LC318R		LC319R		LC320R		LC321R		LC322R		LC323R		LC324R		LC325R		LC326R		LC327R		LC328R		LC329R		LC330R		LC331R		LC332R		LC333R		LC334R		LC335R		LC336R		LC337R		LC338R		LC339R		LC340R		LC341R		LC342R		LC343R		LC344R		LC345R		LC346R		LC347R		LC348R		LC349R		LC350R		LC351R		LC352R		LC353R		LC354R		LC355R		LC356R		LC357R		LC358R		LC359R		LC360R		LC361R		LC362R		LC363R		LC364R		LC365R		LC366R		LC367R		LC368R		LC369R		LC370R		LC371R		LC372R		LC373R		LC374R		LC375R		LC376R		LC377R		LC378R		LC379R		LC380R		LC381R		LC382R		LC383R		LC384R		LC385R		LC386R		LC387R		LC388R		LC389R		LC390R		LC391R		LC392R		LC393R		LC394R		LC395R		LC396R		LC397R		LC398R		LC399R		LC400R		LC401R		LC402R		LC403R		LC404R		LC405R		LC406R		LC407R		LC408R		LC409R		LC410R		LC411R		LC412R		LC413R		LC414R		LC415R		LC416R		LC417R		LC418R		LC419R		LC420R		LC421R		LC422R		LC423R		LC424R		LC425R		LC426R		LC427R		LC428R		LC429R		LC430R		LC431R		LC432R		LC433R		LC434R		LC435R		LC436R		LC437R		LC438R		LC439R		LC440R		LC441R		LC442R		LC443R		LC444R		LC445R		LC446R		LC447R		LC448R		LC449R		LC450R		LC451R		LC452R		LC453R		LC454R		LC455R		LC456R		LC457R		LC458R		LC459R		LC460R		LC461R		LC462R		LC463R		LC464R		LC465R		LC466R		LC467R		LC468R		LC469R		LC470R		LC471R		LC472R		LC473R		LC474R		LC475R		LC476R		LC477R		LC478R		LC479R		LC480R		LC481R		LC482R		LC483R		LC484R		LC485R		LC486R		LC487R		LC488R		LC489R		LC490R		LC491R		LC492R		LC493R		LC494R		LC495R		LC496R		LC497R		LC498R		LC499R		LC500R		LC501R		LC502R		LC503R		LC504R		LC505R		LC506R		LC507R		LC508R		LC509R		LC510R		LC511R		LC512R		LC513R		LC514R		LC515R		LC516R		LC517R		LC518R		LC519R		LC520R		LC521R		LC522R		LC523R		LC524R		LC525R		LC526R		LC527R		LC528R		LC529R		LC530R		LC531R		LC532R		LC533R		LC534R		LC535R		LC536R		LC537R		LC538R		LC539R		LC540R		LC541R		LC542R		LC543R		LC544R		LC545R		LC546R		LC547R		LC548R		LC549R		LC550R		LC551R		LC552R		LC553R		LC554R		LC555R		LC556R		LC557R		LC558R		LC559R		LC560R		LC561R		LC562R		LC563R		LC564R		LC565R		LC566R		LC567R		LC568R		LC569R		LC570R		LC571R		LC572R		LC573R		LC574R		LC575R		LC576R		LC577R		LC578R		LC579R		LC580R		LC581R		LC582R		LC583R		LC584R		LC585R		LC586R		LC587R		LC588R		LC589R		LC590R		LC591R		LC592R		LC593R		LC594R		LC595R		LC596R		LC597R		LC598R		LC599R		LC600R		LC601R		LC602R		LC603R		LC604R		LC605R		LC606R		LC607R		LC608R		LC609R		LC610R		LC611R		LC612R		LC613R		LC614R		LC615R		LC616R		LC617R		LC618R		LC619R		LC620R		LC621R		LC622R		LC623R		LC624R		LC625R		LC626R		LC627R		LC628R		LC629R		LC630R		LC631R		LC632R		LC633R		LC634R		LC635R		LC636R		LC637R		LC638R		LC639R		LC640R		LC641R		LC642R		LC643R		LC644R		LC645R		LC646R		LC647R		LC648R		LC649R		LC650R		LC651R		LC652R		LC653R		LC654R		LC655R		LC656R		LC657R		LC658R		LC659R		LC660R		LC661R		LC662R		LC663R		LC664R		LC665R		LC666R		LC667R		LC668R		LC669R		LC670R		LC671R		LC672R		LC673R		LC674R		LC675R		LC676R		LC677R		LC678R		LC679R		LC680R		LC681R		LC682R		LC683R		LC684R		LC685R		LC686R		LC687R		LC688R		LC689R		LC690R		LC691R		LC692R		LC693R		LC694R		LC695R		LC696R		LC697R		LC698R		LC699R		LC700R		LC701R		LC702R		LC703R		LC704R		LC705R		LC706R		LC707R		LC708R		LC709R		LC710R		LC711R		LC712R		LC713R		LC714R		LC715R		LC716R		LC717R		LC718R		LC719R		LC720R		LC721R		LC722R		LC723R		LC724R		LC725R		LC726R		LC727R		LC728R		LC729R		LC730R		LC731R		LC732R		LC733R		LC734R		LC735R		LC736R		LC737R		LC738R		LC739R		LC740R		LC741R		LC742R		LC743R		LC744R		LC745R		LC746R		LC747R		LC748R		LC749R		LC750R		LC751R		LC752R		LC753R		LC754R		LC755R		LC756R		LC757R		LC758R		LC759R		LC760R		LC761R		LC762R		LC763R		LC764R		LC765R		LC766R		LC767R		LC768R		LC769R		LC770R		LC771R		LC772R		LC773R		LC774R		LC775R		LC776R		LC777R		LC778R		LC779R		LC780R		LC781R		LC782R		LC783R		LC784R		LC785R		LC786R		LC787R		LC788R		LC789R		LC790R		LC791R		LC792R		LC793R		LC794R		LC795R		LC796R		LC797R		LC798R		LC799R		LC800R		LC801R		LC802R		LC803R		LC804R		LC805R		LC806R		LC807R		LC808R		LC809R		LC810R		LC811R		LC812R		LC813R		LC814R		LC815R		LC816R		LC817R		LC818R		LC819R		LC820R		LC821R		LC822R		LC823R		LC824R		LC825R		LC826R		LC827R		LC828R		LC829R		LC830R		LC831R		LC832R		LC833R		LC834R		LC835R		LC836R		LC837R		LC838R		LC839R		LC840R		LC841R		LC842R		LC843R		LC844R		LC845R		LC846R		LC847R		LC848R		LC849R		LC850R		LC851R		LC852R		LC853R		LC854R		LC855R		LC856R		LC857R		LC858R		LC859R		LC860R		LC861R		LC862R		LC863R		LC864R		LC865R		LC866R		LC867R		LC868R		LC869R		LC870R		LC871R		LC872R			

Table 2

Binding specificity of anti-human IL-13 antibodies

	Human IL-13	Human IL-13 variant	Non-human primate IL-13
BAK278D6	+	+	+
BAK502G9	+	+	+
BAK615E3	+	-	-

5

Table 3a

Kinetic analysis of anti-human IL-13 antibodies

IgG	Off-rate (s ⁻¹)	On-rate (M ⁻¹ s ⁻¹)	KD (pM)
BAK278D6	7.41e ⁻³	5.49e ⁵	13500
BAK502G9	4.09e ⁻⁴	2.49e ⁶	178
BAK1167F2	4.05e ⁻⁴	2.99e ⁶	136
BAK1183H4	3.00e ⁻⁴	3.7e ⁶	81

10

Table 3b

Kinetic analysis of anti-murine IL-13 antibodies

IgG	Off-rate (s ⁻¹)	On-rate (M ⁻¹ s ⁻¹)	KD (pM)
BAK209B11	1.98e ⁻²	3.9e ⁶	5100

15

Table 4

Pharmacokinetics of BAK502G9 in 4 allergic but non-challenged cynomolgus primates (2 male/2 female) after a single 10mg/kg 5 i.v bolus dose over 29 days. BAK502G9 levels in serum were measured by ELISA (mean data).

C_{max} ($t=0.25h$) ($\mu\text{g/mL}$)	349.04	
Vd_{inf} ($\text{mL}\cdot\text{kg}^{-1}$)	75.03	<80mL/kg, infers no tissue binding.
C_{linf} ($\text{mL}\cdot\text{hr}^{-1}\cdot\text{kg}^{-1}$)	0.23	
AUC_{inf} ($\text{mg}\cdot\text{h}\cdot\text{mL}^{-1}$)	42.99	
AUC_{ext} (%)	17.34	<30% so clearance and vol. of distribution should be accurate.
$T_{0.5}$ (h)	223.55	

Vd_{inf} = volume of distribution over time 0 - infinity,

10 calculated from the extrapolated AUC.

C_{linf} = clearance over time 0 - infinity, calculated from the extrapolated AUC.

AUC_{inf} = area under the curve (measure of total drug exposure) over time 0 - infinity, including an extrapolated term based 15 on the elimination rate constant (k) and the last observed serum drug concentration.

AUC_{ext} = percentage of the total AUC that is extrapolated.

$T_{0.5}$ = Drug half-life in the terminal elimination phase.

ADDITIONAL STATEMENTS OF INVENTION:

1. An isolated specific binding member for human IL-13, comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the set of CDR's consists of a set of CDR's selected 10 from the group consisting of:

the BAK278D6 set of CDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6,

a set of CDR's which contains one or two amino acid substitutions compared with the BAK278D6 set of CDR's, and 20 each set of CDR's as shown for individual clones in Table 1.

2. An isolated specific binding member according to statement 1 wherein the one or two substitutions are at one or 25 two of the following residues within the CDRs, using the standard numbering of Kabat.

31, 32, 34 in HCDR1

30 52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

56 in LCDR2

95A, 97 in LCDR3

5

3. An isolated specific binding member according to statement 2 wherein the one or two substitutions are made at the following positions from among the identified groups of possible substitute residues for each position:

10

<u>Position of substitution</u>	<u>Substitute Residue selected from the group consisting of</u>
---------------------------------	---

15 31 in HCDR1: Q, D, L, G and E

32 in HCDR1: T

34 in HCDR1: V, I and F

20 52 in HCDR2: D, N, A, R, G and E

52A in HCDR2: D, G, T, P, N and Y

53 in HCDR2: D, L, A, P, T, S, I and R

54 in HCDR2: S, T, D, G, K and I

56 in HCDR2: T, E, Q, L, Y, N, V, A, M and G

25 58 in HCDR2: I, L, Q, S, M, H, D and K

60 in HCDR2: R

61 in HCDR2: R

62 in HCDR2: K and G

64 in HCDR2: R

30 65 in HCDR2: K

96 in HCDR3: R and D

97 in HCDR3: N, D, T and P

98 in HCDR3: R

99 in HCDR3: S, A, I, R, P and K

101 in HCDR3: Y

26 in LCDR1: D and S

5 27 in LCDR1: I, L, M, C, V, K, Y, F, R, T, S, A, H and G

28 in LCDR1: V

30 in LCDR1: G

31 in LCDR1: R

10 56 in LCDR2: T

95A in LCDR3: N

97 in LCDR3: I.

15. 4. An isolated specific binding member according to statement 3 wherein there are two substitutions compared with the BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27.

20 5. An isolated specific binding member according to statement 4 comprising the BAK278D6 set of CDR's with a substitution at HCDR3 residue 99 selected from the group consisting of S, A, I, R, P and K, and/or a substitution at LCDR1 residue 27 selected from the group consisting of I, L, 25 M, C, V, K, Y, F, R, T, S, A, H and G.

6. An isolated specific binding member according to statement 4 comprising the BAK278D6 set of CDR's with S substituted for N at HCDR3 residue 99 and/or I substituted for 30 N at LCDR1 residue 27.

7. An isolated specific binding member according to any one of statements 1 to 6 wherein HCDR1, HCDR2 and HCDR3 of the VH

domain are within a germ-line framework and/or LCDR1, LCDR2 and LCDR3 of the VL domain are within a germ-line framework.

8. An isolated specific binding member according to statement 7 wherein the HCDR1, HCDR2 and HCDR3 of the VH domain are within germ-line framework VH1 DP14.

9. An isolated specific binding member according to statement 7 or statement 8 wherein the HCDR1, HCDR2 and HCDR3 of the VH domain are within germ-line framework VL Vλ3 3h.

10. An isolated specific binding member according to any one of statements 1 to 9 which binds a human IL-13 variant in which arginine at position 130 is replaced by glutamine.

15 11. An isolated specific binding member according to any one of statements 1 to 10 which binds non-human primate IL-13.

20 12. An isolated specific binding member according to statement 11 wherein the non-human primate IL-13 is rhesus or cynomolgus.

25 13. A specific binding member according to any one of statements 8 to 12 comprising the BAK502G9 VH domain (SEQ ID NO: 15).

14. A specific binding member according to any one of statements 8 to 13 comprising the BAK502G9 VL domain (SEQ ID NO: 16).

30 15. A specific binding member according to any one of statements 1 to 14 that binds IL-13 with affinity equal to or better than the affinity of an IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the

BAK502G9 VL domain (SEQ ID NO: 16), the affinity of the specific binding member and the affinity of the antigen-binding site being as determined under the same conditions.

5 16. A specific binding member according to any one of statements 1 to 15 that neutralizes human IL-13.

17. A specific binding member according to statement 16 that neutralizes human IL-13, with a potency equal to or better
10 than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as determined under the same conditions.

15 18. A specific binding member according to any one of statements 1 to 17 that comprises an scFv antibody molecule.

19. A specific binding member according to any one of statements 1 to 17 that comprises an antibody constant region.

20. A specific binding member according to statement 19 that comprises a whole antibody.

25 21. A specific binding member according to statement 20 wherein the whole antibody is IgG4.

22. An isolated antibody VH domain of a specific binding member according to any one of statements 1 to 21.

30 23. An isolated antibody VL domain of a specific binding member according to any one of statements 1 to 21.

24. A composition comprising a specific binding member, antibody VH domain or antibody VL according to any one of statements 1 to 23 and at least one additional component.

5 25. A composition according to statement 24 comprising a pharmaceutically acceptable excipient, vehicle or carrier.

26. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or

10 10 VL domain of a specific binding member according to any one of statements 1 to 23.

27. A host cell *in vitro* transformed with nucleic acid according to statement 26.

15 28. A method of producing a specific binding member or antibody VH or VL domain, the method comprising culturing host cells according to statement 27 under conditions for production of said specific binding member or antibody VH or

20 20 VL domain.

29. A method according to statement 28 further comprising isolating and/or purifying said specific binding member or antibody VH or VL variable domain.

25 30. A method according to statement 28 or statement 29 further comprising formulating the specific binding member or antibody VH or VL variable domain into a composition including at least one additional component.

30 31. A method for producing an antibody antigen-binding domain specific for human IL-13, the method comprising providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid

sequence of a parent VH domain comprising HCDR 1, HCDR2 and HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are the BAK278D6 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, or the BAK502G9 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 7, the HCDR2 has the amino acid sequence of SEQ ID NO: 8, the HCDR3 has the amino acid sequence of SEQ ID NO: 9, a VH domain which is an amino acid sequence variant of the parent VH domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more VH/VL combinations; and

testing said VH domain which is an amino acid sequence variant of the parent VH domain or the VH/VL combination or combinations to identify an antibody antigen binding domain specific for human IL-13.

32. A method according to statement 31 wherein the parent VH domain amino acid sequence is selected from the group consisting of SEQ ID NO: 13 and SEQ ID NO: 15.

33. A method according to statement 31 or statement 32 wherein said one or more VL domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the BAK278D6 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, the LCDR3 has the amino acid sequence of SEQ ID NO: 6, or the BAK502G9 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 10, the LCDR2 has the amino acid sequence of SEQ ID NO: 11, the LCDR3 has the amino acid

sequence of SEQ ID NO: 12, producing one or more VL domains each of which is an amino acid sequence variant of the parent VL domain.

5 34. A method according to statement 33 wherein the parent VL domain amino acid sequence is selected from the group consisting of SEQ ID NO: 14 and SEQ ID NO: 16.

10 35. A method for producing an antibody antigen-binding domain specific for human IL-13, the method comprising

providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VH domain comprising HCDR 1, HCDR2 and HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are 15 the BAK167A11 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 55, the HCDR2 has the amino acid sequence of SEQ ID NO: 56, the HCDR3 has the amino acid sequence of SEQ ID NO: 57, the BAK615E3 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 20 153, the HCDR2 has the amino acid sequence of SEQ ID NO: 154, the HCDR3 has the amino acid sequence of SEQ ID NO: 155, the BAK582F7 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 141, the HCDR2 has the amino acid sequence of SEQ ID NO: 142, the HCDR3 has the amino acid sequence of SEQ ID NO: 25 143, or the BAK612B5 set of HCDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 147, the HCDR2 has the amino acid sequence of SEQ ID NO: 148, the HCDR3 has the amino acid sequence of SEQ ID NO: 149, a VH domain which is an amino acid sequence variant of 30 the parent VH domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more VH/VL combinations; and

testing said VH domain which is an amino acid sequence variant of the parent VH domain or the VH/VL combination or

combinations to identify an antibody antigen binding domain specific for human IL-13.

36. A method according to statement 35 wherein the parent VH domain amino acid sequence is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 33.

37. A method according to statement 35 or statement 36 wherein said one or more VL domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the BAK167A11 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 58, the LCDR2 has the amino acid sequence of SEQ ID NO: 59, the LCDR3 has the amino acid sequence of SEQ ID NO: 60, the BAK615E3 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 156, the LCDR2 has the amino acid sequence of SEQ ID NO: 157, the LCDR3 has the amino acid sequence of SEQ ID NO: 158, the BAK582F7 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 144, the LCDR2 has the amino acid sequence of SEQ ID NO: 145, the LCDR3 has the amino acid sequence of SEQ ID NO: 146, or the BAK612B5 set of LCDR's, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 150, the LCDR2 has the amino acid sequence of SEQ ID NO: 151, the LCDR3 has the amino acid sequence of SEQ ID NO: 152, producing one or more VL domains each of which is an amino acid sequence variant of the parent VL domain.

38. A method according to statement 37 wherein the parent VL domain amino acid sequence is selected from the group consisting of SEQ ID NO: 24 and SEQ ID NO: 34.

39. A method according to any one of statements 31 to 34 wherein said VH domain which is an amino acid sequence variant of the parent VH domain is provided by CDR mutagenesis.

5 40. A method according to any one of statements 35 to 38 wherein said VH domain which is an amino acid sequence variant of the parent VH domain is provided by CDR mutagenesis.

10 41. A method according to any one of statements 31 to 40 further comprising providing the antibody antigen binding site within an IgG, scFv or Fab antibody molecule.

42. A method of producing a specific binding member that binds human IL-13, which method comprises:

15 providing starting nucleic acid encoding a VH domain or a starting repertoire of nucleic acids each encoding a VH domain, wherein the VH domain or VH domains either comprise a HCDR1, HCDR2 and/or HCDR3 to be replaced or lack a HCDR1, HCDR2 and/or HCDR3 encoding region;

20 combining said starting nucleic acid or starting repertoire with donor nucleic acid or donor nucleic acids encoding or produced by mutation of the amino acid sequence of the HCDR1 (SEQ ID NO: 1) or HCDR1 (SEQ ID NO: 7), HCDR2 (SEQ ID NO: 2) or HCDR2 (SEQ ID NO: 8) and/or HCDR3 (SEQ ID NO: 3) or HCDR3 (SEQ ID NO: 9) such that said donor nucleic acid is or donor nucleic acids are inserted into the CDR1, CDR2 and/or CDR3 region in the starting nucleic acid or starting repertoire, so as to provide a product repertoire of nucleic acids encoding VH domains;

25 30 expressing the nucleic acids of said product repertoire to produce product VH domains;

optionally combining said product VH domains with one or more VL domains;

selecting a specific binding member specific for human IL-13, which specific binding member comprises a product VH domain and optionally a VL domain; and

recovering said specific binding member or nucleic acid encoding it.

43. A method according to statement 42 wherein the donor nucleic acids are produced by mutation of said HCDR1 and/or HCDR2.

10 44. A method according to statement 42 wherein the donor nucleic acid is produced by mutation of HCDR3.

15 45. A method according to statement 44 comprising providing the donor nucleic acid by mutation of nucleic acid encoding the amino acid sequence of HCDR3 (SEQ ID NO: 3) or HCDR3 (SEQ ID NO: 9).

20 46. A method according to statement 42 comprising providing the donor nucleic acid by random mutation of nucleic acid.

25 47. A method according to any one of statements 42 to 46 further comprising attaching a product VH domain that is comprised within the recovered specific binding member to an antibody constant region.

48. A method according to any one of statements 42 to 46 comprising providing an IgG, scFv or Fab antibody molecule comprising the product VH domain and a VL domain.

30 49. A method according to any one of statements 31 to 48, further comprising testing the antibody antigen-binding domain or specific binding member that binds human IL-13 for ability to neutralize human IL-13.

50. A method according to statement 49 wherein a specific binding member that comprises an antibody fragment that binds and neutralizes human IL-13 is obtained.

5

51. A method according to statement 50 wherein the antibody fragment is an scFv antibody molecule.

52. A method according to statement 50 wherein the antibody
10 fragment is an Fab antibody molecule.

53. A method according to statement 51 or statement 52 further comprising providing the VH domain and/or the VL domain of the antibody fragment in a whole antibody.

15

54. A method according to any one of statements 31 to 53 further comprising formulating the specific binding member that binds IL-13, antibody antigen-binding site or an antibody VH or VL variable domain of the specific binding member or
20 antibody antigen-binding site that binds IL-13, into a composition including at least one additional component.

55. A method according to any one of statements 31 to 54 further comprising binding a specific binding member that
25 binds human IL-13 to IL-13 or a fragment of IL-13.

56. A method comprising binding a specific binding member that binds IL-13 according to any one of statements 1 to 21 to human IL-13 or a fragment of human IL-13.

30

57. A method according to statement 55 or statement 56 wherein said binding takes place in vitro.

58. A method according to any one of statements 55 to 57 comprising determining the amount of binding of specific binding member to IL-13 or a fragment of IL-13.

5 59. A method according to any one of statements 31 to 58 further comprising use of the specific binding member in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel 10 disease and Hodgkin's lymphoma.

60. Use of a specific binding member according to any one of statements 1 to 21 in the manufacture of a medicament for treatment of a disease or disorder selected from the group 15 consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

61.. A method of treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, 20 allergic rhinitis, fibrosis and Hodgkin's lymphoma, the method comprising administering a specific binding member according to any one of statements 1 to 21 to a patient with the disease or disorder or at risk of developing the disease or disorder.

25 62. An isolated specific binding member for human IL-13, comprising an antibody antigen-binding domain site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises 30 HCDR1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein

HCDR1 is of amino acid sequence which has the formula
HX₁ HX₂ G HX₃ S

wherein

HX₁ is selected from the group consisting of N, Q, D, L, G and E,

HX₂ is selected from the group consisting of Y and T,

5 HX₃ is selected from the group consisting of V, I, F and L,

HCDR2 is of amino acid sequence which has the formula

10 W I HX₄ HX₅ HX₆ HX₇ G HX₈ T HX₉ Y HX₁₀ HX₁₁ HX₁₂ F HX₁₃ HX₁₄

wherein

HX₄ is selected from the group consisting of S, D, N, A, R, G and E,

15 HX₅ is selected from the group consisting of A, D, G, T, P, N and Y,

HX₆ is selected from the group consisting of N, D, L, A, P, T, S, I and R,

HX₇ is selected from the group consisting of N, S, T, D, G, K and I,

20 HX₈ is selected from the group consisting of D, T, E, Q, L, Y, N, V, A, M and G,

HX₉ is selected from the group consisting of N, I, L, Q, S, M, H, D and K,

HX₁₀ is selected from the group consisting of G and R,

25 HX₁₁ is selected from the group consisting of Q and R,

HX₁₂ is selected from the group consisting of E, K and G,

HX₁₃ is selected from the group consisting of Q and R,

HX₁₄ is selected from the group consisting of G and K,

30 HCDR3 is of amino acid sequence which has the formula

D HX₁₅ HX₁₆ HX₁₇ HX₁₈ W A R W HX₁₉ F HX₂₀ L

wherein

HX₁₅ is selected from the group consisting of S, R and D,
HX₁₆ is selected from the group consisting of S, N, D, T

and P,

5 HX₁₇ is selected from the group consisting of S and R,
HX₁₈ is selected from the group consisting of S, N, A, I,
R, P and K,

HX₁₉ is selected from the group consisting of F and Y,

HX₂₀ is selected from the group consisting of D and Y,

10

LCDR1 is of amino acid sequence which has the formula

G G LX₁ LX₂ LX₃ G LX₄ LX₅ L V H

wherein

15

LX₁ is selected from the group consisting of N, D and S,
LX₂ is selected from the group consisting of N, I, L, M,
C, V, K, Y, F, R, T, S, A, H and G,

LX₃ is selected from the group consisting of I and V,

20

LX₄ is selected from the group consisting of S and G,

LX₅ is selected from the group consisting of K and R,

LCDR2 is of amino acid sequence which has the formula

D D G D R P LX₆

25

wherein

LX₆ is selected from the group consisting of S and
T,

30

LCDR3 is of amino acid sequence which has the formula

Q V W D T G S LX₇ P V LX₈

wherein

LX₇ is selected from the group consisting of D and N,
LX₈ is selected from the group consisting of V and I.

63. An isolated specific binding member according to statement
5 62, wherein

HX₁ is selected from the group consisting of D and N,
HX₂ is Y,
HX₃ is L,
10 HX₄ is selected from the group consisting of S and G,
HX₅ is selected from the group consisting of T and A,
HX₆ is N,
HX₇ is selected from the group consisting of N and I,
HX₈ is D,
15 HX₉ is selected from the group consisting of N, D and K,
HX₁₀ is G,
HX₁₂ is selected from the group consisting of E and G,
HX₁₃ is Q,
HX₁₉ is F,
20 LX₁ is selected from the group consisting of N and S,
LX₂ is selected from the group consisting of N, Y, T, S,
and I,
LX₆ is S,
25 LX₇ is D.

64. An isolated specific binding member according to
statement 62, wherein

30 HX₁ is selected from the group consisting of N and D,
HX₂ is Y,
HX₃ is L,
HX₄ is selected from the group consisting of S and G,
HX₅ is selected from the group consisting of A and T,

HX₆ is N,
HX₇ is N,
HX₈ is selected from the group consisting of D and G,
HX₉ is selected from the group consisting of I, S, N and
5 D,
HX₁₁ is Q,
HX₁₂ is E and K,
HX₁₄ is G,
HX₁₅ is S,
10 HX₁₆ is selected from the group consisting of S and N,
HX₁₇ is S,
HX₁₈ is selected from the group consisting of S and N,
HX₁₉ is F,
HX₂₀ is D,
15 LX₁ is selected from the group consisting of N and D,
LX₃ is I,
LX₈ is V.
20 65. An isolated specific binding member according to
statement 62, wherein
HX₇ is selected from the group consisting of N, S, T, D,
G and K,
25 HX₈ is selected from the group consisting of D, T, E, Q,
L, Y, N, V, A, M,
HX₉ is selected from the group consisting of N, I, L, Q,
S, M and H,
HX₁₀ is G,
30 HX₁₁ is Q,
HX₁₂ is F,
HX₁₃ is Q,
HX₁₄ is G,
HX₁₅ is S,

HX₁₆ is selected from the group consisting of N and S,
HX₁₇ is S,

HX₁₈ is selected from the group consisting of N and S,
HX₁₉ is F,

5 HX₂₀ is D,

LX₁ is N,

LX₂ is selected from the group consisting of N and I,

LX₃ is I,

10 LX₄ is S,

LX₅ is K,

LX₆ is S,

LX₇ is D,

LX₈ is V.

15

66. An isolated specific binding member according to
statement 65, wherein

HX₁ is selected from the group consisting of N, Q and D,

20 HX₃ is selected from the group consisting of L, V and I,

HX₄ is selected from the group consisting of S, N, A and
R,

HX₅ is selected from the group consisting of A, D, T, G,
N and Y,

25 HX₆ is selected from the group consisting of N, A, P, S,
D and I,

HX₇ is selected from the group consisting of N, T, D and
G,

30 HX₈ is selected from the group consisting of D, Q, Y and
N,

HX₉ is selected from the group consisting of N, Q, S and
I.

67. A specific binding member according to any one of statements 62 to 66 that neutralizes human IL-13.

68. A specific binding member according to statement 67 that 5 neutralizes human IL-13, with a potency equal to or better than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as 10 determined under the same conditions.

69. A specific binding member according to any one of statements 62 to 68 that comprises an scFv antibody molecule.

70. A specific binding member according to any one of statements 62 to 68 that comprises an antibody constant region. 15

71. A specific binding member according to statement 70 that 20 comprises a whole antibody.

72. A specific binding member according to statement 71 wherein the whole antibody is IgG4.

73. An isolated specific binding member according to any one of statements 62 to 72 which binds a human IL-13 variant in which arginine at position 130 is replaced by glutamine. 25

74. An isolated specific binding member according to any one of statements 62 to 72 which binds non-human primate IL-13. 30

75. An isolated specific binding member according to statement 74 wherein the non-human primate IL-13 is rhesus or cynomolgus.

76. An isolated antibody VH domain of a specific binding member according to any one of statements 62 to 75.

5 77. An isolated antibody VL domain of a specific binding member according to any one of statements 62 to 75.

10 78. A composition comprising the specific binding member, antibody VH domain or antibody VL domain of any one of statements 62 to 77 and at least one additional component.

15 80. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or VL domain of a specific binding member according to any one of statements 62 to 77.

20 81. A host cell *in vitro* transformed with nucleic acid according to statement 80.

25 82. A method of producing a specific binding member or antibody VH or VL domain, the method comprising culturing host cells according to statement 81 under conditions for production of said specific binding member or antibody VH or VL domain.

30 83. A method according to statement 82 further comprising isolating and/or purifying said specific binding member or antibody VH or VL variable domain.

84. A method according to statement 82 or statement 83 further comprising formulating the specific binding member or

antibody VH or VL variable domain into a composition including at least one additional component.

85. A method according to any one of statements 82 to 84
5 further comprising binding a specific binding member that binds human IL-13 to IL-13 or a fragment of IL-13.

86. A method comprising binding a specific binding member that binds IL-13 according to any one of statements 62 to 75
10 to human IL-13 or a fragment of human IL-13.

87. A method according to statement 85 or statement 86 wherein said binding takes place *in vitro*.

15 88. A method according to any one of statements 85 to 87 comprising determining the amount of binding of specific binding member to IL-13 or a fragment of IL-13.

89. A method according to any one of statements 82 to 84
20 further comprising use of the specific binding member in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

25 90. Use of a specific binding member according to any one of statements 62 to 75 in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

91. A method of treatment of a disease or disorder selected from the group consisting of asthma, atopic dermatitis, allergic rhinitis, fibrosis and Hodgkin's lymphoma, the method

comprising administering a specific binding member according to any one of statements 62 to 75 to a patient with the disease or disorder or at risk of developing the disease or disorder.

BAK278D6

HEAVY CHAIN

CDR1- SEQ ID NO 1: NYGLS
5 CDR2- SEQ ID NO 2: WISANGDTNYGQEFQG
CDR3- SEQ ID NO 3: DSSSNWARWFFDL

BAK278D6

10 LIGHT CHAIN

CDR1- SEQ ID NO 4: GGNNIGSKLVH
CDR2- SEQ ID NO 5: DDGDRPS
CDR3- SEQ ID NO 6: QVWDTGSDPVV

15

BAK502G9

HEAVY CHAIN

20 CDR1-SEQ ID NO 7: NYGLS
CDR2-SEQ ID NO 8: WISANGDTNYGQEFQG
CDR3-SEQ ID NO 9: DSSSSWARWFFDL

LIGHT CHAIN

25

CDR1-SEQ ID NO 10: GGNIIGSKLVH
CDR2-SEQ ID NO 11: DDGDRPS
CDR3-SEQ ID NO 12: QVWDTGSDPVV

30

BAK278D6

HEAVY CHAIN DOMAIN

SEQ ID NO 13:

EVQLVQSGAEVKKPGASVKVSCKASGYTFRNYGLSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRITMTTETSTNTAHMELRSLRSDDTAVYYCVRDSSNWARWFFDLWGKGTMVTVSS

BAK278D6

5 LIGHT CHAIN DOMAIN

SEQ ID NO 14:

SYVLTQPPSVSVA
PGQTARI
PCGGNNIGSKLVH
WYQQKPGQAPV
LVVYDDGDRPS
GIPERFS
GSNSGNTATLT
ISRIDAGDEAD
YYCQVWDTGSD
PVVFGGGTKL
TVL

10

BAK502G9

15 HEAVY CHAIN DOMAIN

SEQ ID NO 15:

QVQLVQSGAEVKKPGASVKVSCKASGYTFT
NYGLSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAY
MELRSLRSDDTAVYY
CARDSSSSWARWFFDLWGR
GTLTVSS

20

BAK502G9

LIGHT CHAIN DOMAIN

SEQ ID NO 16:

SYVLTQPPSVSVA
PGKTARI
TCGGNIIGSKLVH
WYQQKPGQAPV
LVVIYDDGDRPS
GIPERFS
GSNSGNTATLT
ISRVEAGDEAD
YYCQVWDTGSD
PVVFGGGTKL
TVL

BAK278D6

30 HEAVY CHAIN

FR1- SEQ ID NO 17: EVQLVQSGAEVKKPGASVKVSCKASGYTFR

FR2- SEQ ID NO 18: WVRQAPGQGLEWMG

FR3- SEQ ID NO 19: RITMTTETSTNTAHMELRSLRSDDTAVYYCVR

BAK278D6**LIGHT CHAIN**

5 FR1- SEQ ID NO 20: SYVLTQPPSVSVAPGQTARI

FR2- SEQ ID NO 21: WYQQKPGQAPVLVVY

FR3- SEQ ID NO 22: GIPERFSGSNSGNTATLTISRIDAGDEADYYC

BAK167A11**10 HEAVY CHAIN DOMAIN**

SEQ ID NO 23:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGAAGEGYYGYWGRGTLVTVSS

15

BAK167A11**LIGHT CHAIN DOMAIN**

SEQ ID NO 24:

20 NFMLTQPHSVSESPGKTVTISCTRSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGV PDR
FSGSIDSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGKT VTVL**BAK209B11****HEAVY CHAIN DOMAIN**

25

SEQ ID NO 25:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSSYGM SWVRQAPGKGLEWVSSISASGDSTFYA
DSVKGRFTISRDNNKNMVFLQVNSLRADDTAVYFCAKDWSQWL VGDADF DVWGRGTTVTVSS30 **BAK209B11****LIGHT CHAIN DOMAIN**

SEQ ID NO 26:

DIQLTQSPSTLSASVGDRVITCRASQSVSLWVAWYQQRPGKAPKLLIYDGSTLQSGVPARF
SGSGSGTEFTLTISLQPDDFATYYCQQYKTFSTFGQGTKVEIKRA

BAK502G9

5 HEAVY CHAIN

FR1- SEQ ID NO 27: QVQLVQSGAEVKKPGASVKVSCKASGYTFT

FR2- SEQ ID NO 28: WYRQAPGQGLEWMG

FR3- SEQ ID NO 29: RVTMTTDSTSTAYMELRSLRSDDTAVYYCAR

10

BAK502G9

LIGHT CHAIN

FR1- SEQ ID NO 30: SYVLTQPPSVSVPAGKTARITC

15 FR2- SEQ ID NO 31: WYQQKPGQAPVLVIY

FR3- SEQ ID NO 32: GIPERFSGNSGNTATLTISRVEAGDEADYYC

BAK615E3

HEAVY CHAIN DOMAIN

20

SEQ ID NO 33:

EVQLLESGGGLVQPQGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGKATTEEGYYGYWGRGTLVTVSS

25 ***BAK615E3***

LIGHT CHAIN DOMAIN

SEQ ID NO 34:

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGVPDF

30 FSGSIDSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGGTKVTVL

5 **BAK1167F2**

HEAVY CHAIN DOMAIN

SEQ ID NO 35:

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFEQTGVSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSWARWFFDLWGRGTLTVSS

BAK1167F2

LIGHT CHAIN DOMAIN

15

SEQ ID NO 36:

SYVLTQPPSVSVPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

20

BAK1183H4

HEAVY CHAIN DOMAIN

SEQ ID NO 37:

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMWINYDGGNTQYQGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSWARWFFDLWGRGTLTVSS

BAK1183H4

LIGHT CHAIN DOMAIN

30

SEQ ID NO 38:

SYVLTQPPSVSVPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1105H3**HEAVY CHAIN DOMAIN****SEQ ID NO 39:**

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISGLNGETLYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLVTVSS

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BAK1105H3**LIGHT CHAIN DOMAIN****SEQ ID NO 40:**

15 SYVLTQPPSVSVPGKTARITCGGANIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGKLTVL

BAK1111D1020 **HEAVY CHAIN DOMAIN****SEQ ID NO 41:**

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIATPDGQTSYQG
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSNSWARWFFDLWGRGTLVTVSS

25

BAK1111D10**LIGHT CHAIN DOMAIN**30 **SEQ ID NO 42:**

SYVLTQPPSVSVPGKTARITCGGANIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGKLTVL

BAK1167F4

HEAVY CHAIN DOMAIN

SEQ ID NO 43:

5. QVQLVQSGAEVKKPGASVKVSCKASGYTFIDTGVSWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1167F4

LIGHT CHAIN DOMAIN

10

SEQ ID NO 44:

SYVLTQPPSVSVPKGKARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGKLTVL

15

BAK1184C8

HEAVY CHAIN DOMAIN

SEQ ID NO 45:

20 QVQLVQSGAEVKKPGASVKVSCKASGYFTNYGLSWVRQAPGQGLEWMGWISGSNGYTSYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1184C8

LIGHT CHAIN DOMAIN

25

SEQ ID NO 46:

SYVLTQPPSVSVPKGKARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGKLTVL

30

BAK1185E1

HEAVY CHAIN DOMAIN

SEQ ID NO 47:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWINDATGDTQYQQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1185E1

5 LIGHT CHAIN DOMAIN

SEQ ID NO 48:

SYVLTQPPSVSVPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

10

BAK1185F8

HEAVY CHAIN DOMAIN

15 SEQ ID NO 49:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGLSWVRQAPGQGLEWMGWIRNIDGYTIYQG
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1185F8

20 LIGHT CHAIN DOMAIN

SEQ ID NO 50:

SYVLTQPPSVSVPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

25

BAK1187B4

HEAVY CHAIN DOMAIN

SEQ ID NO 51:

30 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIDDSGTTIYQG
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1187B4

LIGHT CHAIN DOMAIN

SEQ ID NO 52:

SYVLTQPPSVSVPKGKARITCGGNIIGSKLVHWWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

5

BAK1166G2

HEAVY CHAIN DOMAIN

10 SEQ ID NO 53:

QVQLVQSGAEVKKPGASVKVSCKASGYTFANTGISWVRQAPGQGLEWMGWISANNGDTNYGQ
EFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLTVSS

BAK1166G2

15 LIGHT CHAIN DOMAIN

SEQ ID NO 54:

SYVLTQPPSVSVPKGKARITCGGNIIGSKLVHWWYQQKPGQAPVLVIYDDGDRPSGIPERFS
GSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

20

BAK167A11

HEAVY CHAIN

25 CDR1- SEQ ID NO 55: SYAMS

CDR2- SEQ ID NO 56: AISGSGGSTYYADSVKG

CDR3- SEQ ID NO 57: VGAAGEGYYGY

BAK167A11

30 LIGHT CHAIN

CDR1- SEQ ID NO 58: TRSSGSIASNYVQ

CDR2- SEQ ID NO 59: DDNQRPS

CDR3- SEQ ID NO 60: QSYDSNNDV

BAK1167F2**HEAVY CHAIN**

5 CDR1- SEQ ID NO 61: QTGVS
CDR2- SEQ ID NO 62: WISANGDTNYGQEFQG
CDR3- SEQ ID NO 63: DSSSSWARWFFDL

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BAK1167F2**LIGHT CHAIN**

15 CDR1- SEQ ID NO 64: GGNIIGSKLVH
CDR2- SEQ ID NO 65: DDGDRPS
CDR3- SEQ ID NO 66: QVWDTGSDPVV

BAK1166G220 **HEAVY CHAIN**

CDR1- SEQ ID NO 67: NTGIS
CDR2- SEQ ID NO 68: WISANGDTNYGQEFQG
CDR3- SEQ ID NO 69: DSSSSWARWFFDL

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BAK1166G2**LIGHT CHAIN**

CDR1- SEQ ID NO 70: GGNIIGSKLVH
30 CDR2- SEQ ID NO 71: DDGDRPS
CDR3- SEQ ID NO 72: QVWDTGSDPVV

BAK1184C8**HEAVY CHAIN**

CDR1- SEQ ID NO 73: NYGLS
CDR2- SEQ ID NO 74: WISGNGYTSYGKEFQG
CDR3- SEQ ID NO 75: DSSSSWARWFFDL

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BAK1184C8

LIGHT CHAIN

CDR1- SEQ ID NO 76: GGNIIGSKLVH
10 CDR2- SEQ ID NO 77: DDGDRPS
CDR3- SEQ ID NO 78: QVWDTGSDPVV

BAK1185E1

HEAVY CHAIN

15

CDR1- SEQ ID NO 79: NYGLS
CDR2- SEQ ID NO 80: WINDTGDTQYGQEFGQG
CDR3- SEQ ID NO 81: DSSSSWARWFFDL

20 *BAK1185E1*

LIGHT CHAIN

CDR1- SEQ ID NO 82: GGNIIGSKLVH
CDR2- SEQ ID NO 83: DDGDRPS
25 CDR3- SEQ ID NO 84: QVWDTGSDPVV

BAK1167F4

HEAVY CHAIN

30 CDR1- SEQ ID NO 85: DTGVS
CDR2- SEQ ID NO 86: WISANGDTNYGQEFGQG
CDR3- SEQ ID NO 87: DSSSSWARWFFDL

BAK1167F4**LIGHT CHAIN**

CDR1- SEQ ID NO 88: GGNIIGSKLVH
5 CDR2- SEQ ID NO 89: DDGDRPS
CDR3- SEQ ID NO 90: QVWDTGSDPVV

BAK1111D10**HEAVY CHAIN**

10 CDR1- SEQ ID NO 91: NYGLS
CDR2- SEQ ID NO 92: WIATDGQTSYQGEFQG
CDR3- SEQ ID NO 93: DSSSSWARWFFDL

15 BAK1111D10**LIGHT CHAIN**

CDR1- SEQ ID NO 94: GGNIIGSKLVH
CDR2- SEQ ID NO 95: DDGDRPS
20 CDR3- SEQ ID NO 96: QVWDTGSDPVV

BAK1183H4**HEAVY CHAIN**

25 CDR1- SEQ ID NO 97: NYGLS
CDR2- SEQ ID NO 98: WINYGGNTQYQGEFQG
CDR3- SEQ ID NO 99: DSSSSWARWFFDL

BAK1183H4**30 LIGHT CHAIN**

CDR1- SEQ ID NO 100: GGNIIGSKLVH
CDR2- SEQ ID NO 101: DDGDRPS
CDR3- SEQ ID NO 102: QVWDTGSDPVV

BAK1185H8**HEAVY CHAIN**

5 CDR1- SEQ ID NO 103: DYGLS

CDR2- SEQ ID NO 104: WRINDGYTIYGQEFGQ

CDR3- SEQ ID NO 105: DSSSSWARWFFDL

BAK1185H810 **LIGHT CHAIN**

CDR1- SEQ ID NO 106: GGNIIGSKLVH

CDR2- SEQ ID NO 107: DDGDRPS

CDR3- SEQ ID NO 108: QVWDTGSDPVV

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BAK278D6**HEAVY CHAIN- SEQ ID NO: 109**20 CAGGTGCAGCTGGTGCAGTCAGGCTGAGGTGAAGAACGCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCACT
25 GGGCCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA**BAK278D6****LIGHT CHAIN- SEQ ID NO:110**30 TCCTATGTGCTGACTCAGCCACCCCTGGTGTAGTGGCCCCAGGTAAGACGGCCAGGATTAC
CTGTGGGGAAACAACATTGGAAGTAAACTTGTACACTGGTACCGAGCAGAACGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTGGAACACGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC

CGACTATTATTGTCAAGGTGTGGATACTGGTAGTGAATCCCCTGGTATTGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

5 **BAK502G9**

HEAVY CHAIN- SEQ ID NO:111

10 CAGGTCCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCAGCGCTAACATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

15 **BAK502G9**

LIGHT CHAIN- SEQ ID NO:112

20 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGAAACATCATTGAAAGTAAACTGTACACTGGTACCGAGCAGGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTGGAACACGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAAGGTGTGGATACTGGTAGTGAATCCCCTGGTATTGGCGGAGGGACCA
AGCTGACCGTCCTAGGT

25 **BAK1105H03**

HEAVY CHAIN- SEQ ID NO: 113

30 CAGGTCCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCTCCGGCTTGAACGGCGAGACATTGTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

BAK1105H03

LIGHT CHAIN- SEQ ID NO: 114

TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
5 CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAACGCCAGG
CCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTCTGGAACACGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAGGTGTGGATACTGGTAGTGATCCCGTGGTATTGGCGAGGGACCA
AGCTGACCGTCCTAGGT

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BAK1111D10

HEAVY CHAIN- SEQ ID NO:115

CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAACGCTGGGCCCTCAGTGAAGGTCTC
15 CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATCGCAACCCAGACGCCAGACAAGCTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAACAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGACACTGGTCACCGTCTCCTCA

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BAK1111D10

LIGHT CHAIN- SEQ ID NO:116

25 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAACGCCAGG
CCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTCTGGAACACGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAGGTGTGGATACTGGTAGTGATCCCGTGGTATTGGCGAGGGACCA
30 AGCTGACCGTCCTAGGT

BAK 1167F2

HEAVY CHAIN- SEQ ID NO: 117

CAAGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
 CTGCAAGGCTTCTGGTTACACCTTGAGCAGACCGCGTCTCCTGGTGCACAGGCCCTG
 GACAAGGGCTTGAGTGGATGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
 GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
 5 GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
 GGGCCCGCTGGTTTCGATCTCTGGGGCGGGGACACTGGTCACCGTCTCCTCA

BAK 1167F2

LIGHT CHAIN- SEQ ID NO:118

10 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGCCAGGATTAC
 CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCGAGCAGGCCAGGCCAGG
 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
 GGCTCCAACCTCTGGAACACGCCACCCCTGACCATCAGCAGGTCGAGGCCGGGATGAGGC
 15 CGACTATTATTGTCAGGTGTGGATACTGGTAGTGTGATCCGTGGTATTGGCGAGGGACCA
 AGCTGACCGTCCTAGGT

BAK 1167F04

HEAVY CHAIN- SEQ ID NO:119

20 CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
 CTGCAAGGCTTCTGGTTACACCTTATGACACCGGGTCTCCTGGTGCACAGGCCCTG
 GACAAGGGCTTGAGTGGATGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
 GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
 25 GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
 GGGCCCGCTGGTTTCGATCTCTGGGGCGGGGACACTGGTCACCGTCTCCTCA

BAK 1167F04

LIGHT CHAIN- SEQ ID NO:120

30 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGCCAGGATTAC
 CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCGAGCAGGCCAGGCCAGG
 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
 GGCTCCAACCTCTGGAACACGCCACCCCTGACCATCAGCAGGTCGAGGCCGGGATGAGGC

CGACTATTATTGTCAAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTGGCGGGAGGGACCA
AGCTGACCGTCCTAGGT

5 **BAK 1183H4**

HEAVY CHAIN- SEQ ID NO:121

10 CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCAACTACGACGGCGAACACACAGTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

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BAK 1183H4

LIGHT CHAIN- SEQ ID NO:122

20 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCGAGCAGAACGCCAGGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTGGAACACGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAAGGTGTGGATACTGGTAGTGATCCCGTGGTATTGGCGGGAGGGACCA
AGCTGACCGTCCTAGGT

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BAK1184C8

HEAVY CHAIN- SEQ ID NO:123

30 CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGGTGCGACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCAGCGGGAGCAACGGCTACACATCTTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

BAK1184C8

LIGHT CHAIN- SEQ ID NO:124

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TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
 CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
 GGCTCCAACCTCTGGAACACGGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
 10 CGACTATTATTGTCAGGTGTGGATACTGGTAGTGATCCGTGGTATTGGCGGAGGGACCA
 AGCTGACCGTCCTAGGT

BAK1185E1

15 HEAVY CHAIN- SEQ ID NO:125

CAGGTGCAGCTGGTGCAGTCGGGCTGAGGTGAAGAACGCTGGGCCTCAGTGAAGGTCTC
 CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGTGCACAGGCCCTG
 GACAAGGGCTTGAGTGGATGGATGGATCAACGACGCCACCGCGACACACAGTATGGACAG
 20 GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
 GAGGAGCCTGAGATCTGACGACACGGCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
 GGGCCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

BAK1185E1

25 LIGHT CHAIN- SEQ ID NO:126

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
 CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
 30 GGCTCCAACCTCTGGAACACGGCCACCCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
 CGACTATTATTGTCAGGTGTGGATACTGGTAGTGATCCGTGGTATTGGCGGAGGGACCA
 AGCTGACCGTCCTAGGT

BAK1185F8**HEAVY CHAIN- SEQ ID NO:127**

5 CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAGATTATGGTCTCAGCTGGTGCACAGGCCCTG
GACAAGGGCTAGAGTGGATGGATCCGAAACATCGACGGCTACACAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

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BAK1185F8**LIGHT CHAIN- SEQ ID NO:128**

15 TCCTATGTGCTGACTCAGCCACCCTCGGTGTCACTGGCCCCAGGAAAGACGCCAGGATTAC
CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCGAGCAGAACGCCAGG
CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTGGAACACGCCACCCCTGACCATCAGCAGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAGGTGTGGATACTGGTAGTGTACCCGTGGTATTGGCGAGGGACCA
AGCTGACCGTCCTAGGT

20

BAK1187B4**HEAVY CHAIN- SEQ ID NO:129**

25 CAGGTCCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTCAGTGAAGGTCTC
CTGCAAGGCTTCTGGTTACACCTTACAAATTATGGTCTCAGCTGGTGCACAGGCCCTG
GACAAGGGCTTGAGTGGATGGATGGATCGACGACAGCAGCACGACAATATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
30 GGGCCCGCTGGTTTCGATCTCTGGGCCGGGGACACTGGTCACCGTCTCCTCA

BAK1187B4

LIGHT CHAIN- SEQ ID NO:130

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCACTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
5 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTCTGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAGGTGTGGATACTGGTAGTGTACCGTGGTATTGGCGAGGGACCA
AGCTGACCGTCCTAGGT

10 BAK1166G02

HEAVY CHAIN- SEQ ID NO: 131

CAAGTGCAGTTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCCTCAGTGAAGGTCTC
CTGCAAGGCCTCTGGTTACACCTTGCGAACACCCGGATCTCGTGGGTGCGACAGGCCCTG
15 GACAAGGGCTTGAGTGGATGGATGGATCAGCGCTAATAATGGCGACACAAATTATGGACAG
GAATTCCAGGGCAGAGTCACCATGACCACAGATACTCCACGAGCACAGCCTACATGGAGTT
GAGGAGCCTGAGATCTGACGACACGCCGTTATTACTGTGCGAGAGACTCCAGCAGCAGCT
GGGCCGCTGGTTTCGATCTGGGTGGGACACTGGTACCGTCTCCTCA

20 BAK1166G02

LIGHT CHAIN- SEQ ID NO:132

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCACTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAGGCCAGG
25 CCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACCTCTGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCCGGGATGAGGC
CGACTATTATTGTCAGGTGTGGATACTGGTAGTGTACCGTGGTATTGGCGAGGGACCA
AGCTGACCGTCCTAGGT

30

BAK165E7

HEAVY CHAIN- SEQ ID NO: 133

EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISANNGETNYGQ
EFQGRVTMTTETPTNTAHMELRSLTSDDTAVYYCVRDSSSNWARWYFDLWGQGTLTVSS

BAK165E7

5 LIGHT CHAIN- SEQ ID NO: 134

SYVLTQPPSVSVAPGQTARI PCGGNNIGSKLVH WYQQKPGQAPV LVVYDDGDRPSGIPERFS
GSNSGNTATLTISRIDAGDEADYYCQVWDTGS D P V V F G G G T K L T V L G

10

BAK165E7

HEAVY CHAIN

15

CDR1- SEQ ID NO:135 NYGLS

CDR2- SEQ ID NO:136 WISANNGETNYGQE FQG

CDR3- SEQ ID NO:137 DSSSNWARWYFDL

20 **BAK165E7**

LIGHT CHAIN

CDR1- SEQ ID NO:138 GGNNIGSKLVH

CDR2- SEQ ID NO:139 DDGDRPS

25 CDR3- SEQ ID NO:140 QVWDTGS D P V V

BAK582F7

HEAVY CHAIN

30 CDR1- SEQ ID NO 141: SYAMS

CDR2- SEQ ID NO 142: AISGSGGSTYYADSVKG

CDR3- SEQ ID NO 143: VGAAGEGYYGY

BAK582F7

LIGHT CHAIN

CDR1-SEQ ID NO 144: TRSSGSIASNYVE

CDR2-SEQ ID NO 145: DDNQRPS

5 CDR3-SEQ ID NO 146: QSYDSNNDV

BAK612B5

HEAVY CHAIN

10 CDR1- SEQ ID NO 147: SYAMS

CDR2- SEQ ID NO 148: AISGGGGSTYYADSVKG

CDR3- SEQ ID NO 149: VGRATTDEGYYGY

BAK612B5

15 LIGHT CHAIN

CDR1- SEQ ID NO 150: TRSSGSIASNYVQ

CDR2- SEQ ID NO 151: DDNQRPS

CDR3- SEQ ID NO 152: QSYDSNNDV

20

BAK615E3

25 HEAVY CHAIN

CDR1- SEQ ID NO 153: SYAMS

CDR2- SEQ ID NO 154: AISGGGGSTYYADSVKG

CDR3- SEQ ID NO 155: VGKATTEEGYY

30

BAK615E3

LIGHT CHAIN

CDR1- SEQ ID NO 156: TRSSGSIASNYVQ

CDR2- SEQ ID NO 157: DDNQRPS

CDR3- SEQ ID NO 158: QSYDSNNDV

5 **BAK0278D6**

HEAVY CHAIN

CDR1- SEQ ID NO 159: AATTATGGTCTCAGC

CDR2- SEQ ID NO 160: TGGATCAGCGCTAATAATGGCGACACAAATTAT
10 GGACAGGAATTCCAGGGC

CDR3- SEQ ID NO 161: GACTCCAGCAGCAACTGGGCCGCTGGTTTTTC
GATCTC

BAK278D6

LIGHT CHAIN

15

CDR1- SEQ ID NO 162: GGGGGAAACAAACATTGGAAGTAAACTTGTACAC

CDR2- SEQ ID NO 163: GATGATGGCGACCGGCCCTCA

CDR3- SEQ ID NO 164: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

20

BAK502G9

HEAVY CHAIN

CDR1- SEQ ID NO 165: AATTATGGTCTCAGC

25 CDR2- SEQ ID NO 166: TGGATCAGCGCTAATAATGGCGACACAAATTATGGACA
GGAATTCCAGGGC

CDR3- SEQ ID NO 167: GACTCCAGCAGCAGCTGGGCCGCTGGTTTCGATCTC

BAK502G9

30 LIGHT CHAIN

CDR1- SEQ ID NO 168: GGGGGAAACATCATTGGAAGTAAACTTGTACAC

CDR2- SEQ ID NO 169: GATGATGGCGACCGGCCCTCA

CDR3- SEQ ID NO 170: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

CH Domains- SEQ ID NO: 171

5 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFP
YSLSSVTVPSLGTKYTCNVVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
10 TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPVYTLPPSQEEMTKNQVSLTCL
10 VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNHYTQKSLSLSLGK

CL Domain- SEQ ID NO: 172

15 QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSN
NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

CLAIMS:

1. An isolated specific binding member for human IL-13, comprising an antibody antigen-binding domain site which is
5 composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDR's, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein

10

HCDR1 is of amino acid sequence which has the formula

HX₁ HX₂ G HX₃ S

wherein

HX₁ is selected from the group consisting of N, Q, D, L, G
15 and E,

HX₂ is selected from the group consisting of Y and T,

HX₃ is selected from the group consisting of V, I, F and
L,

20 HCDR2 is of amino acid sequence which has the formula

W I HX₄ HX₅ HX₆ HX₇ G HX₈ T HX₉ Y HX₁₀ HX₁₁ HX₁₂ F HX₁₃ HX₁₄

wherein

HX₄ is selected from the group consisting of S, D, N, A,
25 R, G and E,

HX₅ is selected from the group consisting of A, D, G, T,
P, N and Y,

HX₆ is selected from the group consisting of N, D, L, A,
P, T, S, I and R,

30 HX₇ is selected from the group consisting of N, S, T, D,
G, K and I,

HX₈ is selected from the group consisting of D, T, E, Q,
L, Y, N, V, A, M and G,

HX₉ is selected from the group consisting of N, I, L, Q, S, M, H, D and K,

HX₁₀ is selected from the group consisting of G and R,

HX₁₁ is selected from the group consisting of Q and R,

5 HX₁₂ is selected from the group consisting of E, K and G,

HX₁₃ is selected from the group consisting of Q and R,

HX₁₄ is selected from the group consisting of G and K,

HCDR3 is of amino acid sequence which has the formula

10

D HX₁₅ HX₁₆ HX₁₇ HX₁₈ W A R W HX₁₉ F HX₂₀ L

wherein

15 HX₁₅ is selected from the group consisting of S, R and D,

HX₁₆ is selected from the group consisting of S, N, D, T

and P,

HX₁₇ is selected from the group consisting of S and R,

HX₁₈ is selected from the group consisting of S, N, A, I,

20 R, P and K,

HX₁₉ is selected from the group consisting of F and Y,

HX₂₀ is selected from the group consisting of D and Y,

LCDR1 is of amino acid sequence which has the formula

25

G G LX₁ LX₂ LX₃ G LX₄ LX₅ L V H

wherein

30 LX₁ is selected from the group consisting of N, D and S,

LX₂ is selected from the group consisting of N, I, L, M,

C, V, K, Y, F, R, T, S, A, H and G,

LX₃ is selected from the group consisting of I and V,

LX₄ is selected from the group consisting of S and G,

LX₅ is selected from the group consisting of K and R,

LCDR2 is of amino acid sequence which has the formula

D D G D R P LX₆

5 wherein

LX₆ is selected from the group consisting of S and T,

LCDR3 is of amino acid sequence which has the formula

10

Q V W D T G S LX₇ P V LX₈

wherein

LX₇ is selected from the group consisting of D and N,

15

LX₈ is selected from the group consisting of V and I.

2. An isolated specific binding member according to claim 1, wherein

20

HX₁ is selected from the group consisting of D and N,

HX₂ is Y,

HX₃ is L,

HX₄ is selected from the group consisting of S and G,

HX₅ is selected from the group consisting of T and A,

25

HX₆ is N,

HX₇ is selected from the group consisting of N and I,

HX₈ is D,

HX₉ is selected from the group consisting of N, D and K,

HX₁₀ is G,

30

HX₁₂ is selected from the group consisting of E and G,

HX₁₃ is Q,

HX₁₉ is F,

LX₁ is selected from the group consisting of N and S,

LX₂ is selected from the group consisting of N, Y, T, S, and I,
LX₆ is S,
LX₇ is D.

5

3. An isolated specific binding member according to claim 1, wherein

HX₁ is selected from the group consisting of N and D,
10 HX₂ is Y,
HX₃ is L,
HX₄ is selected from the group consisting of S and G,
HX₅ is selected from the group consisting of A and T,
HX₆ is N,
15 HX₇ is N,
HX₈ is selected from the group consisting of D and G,
HX₉ is selected from the group consisting of I, S, N and
D,
HX₁₁ is Q,
20 HX₁₂ is E and K,
HX₁₄ is G,
HX₁₅ is S,
HX₁₆ is selected from the group consisting of S and N,
HX₁₇ is S,
25 HX₁₈ is selected from the group consisting of S and N,
HX₁₉ is F,
HX₂₀ is D,

LX₁ is selected from the group consisting of N and D,
30 LX₃ is I,
LX₈ is V.

4. An isolated specific binding member according to claim 1, wherein

HX₇ is selected from the group consisting of N, S, T, D, G and K,

HX₈ is selected from the group consisting of D, T, E, Q, 5 L, Y, N, V, A, M,

HX₉ is selected from the group consisting of N, I, L, Q, S, M and H,

HX₁₀ is G,

HX₁₁ is Q,

10 HX₁₂ is F,

HX₁₃ is Q,

HX₁₄ is G,

HX₁₅ is S,

HX₁₆ is selected from the group consisting of N and S,

15 HX₁₇ is S,

HX₁₈ is selected from the group consisting of N and S,

HX₁₉ is F,

HX₂₀ is D,

20 LX₁ is N,

LX₂ is selected from the group consisting of N and I,

LX₃ is I,

LX₄ is S,

LX₅ is K,

25 LX₆ is S,

LX₇ is D,

LX₈ is V.

5. An isolated specific binding member according to claim 4, 30 wherein

HX₁ is selected from the group consisting of N, Q and D,

HX₃ is selected from the group consisting of L, V and I,

HX₄ is selected from the group consisting of S, N, A and R,

HX₅ is selected from the group consisting of A, D, T, G, N and Y,

5 HX₆ is selected from the group consisting of N, A, P, S, D and I,

HX₇ is selected from the group consisting of N, T, D and G,

10 HX₈ is selected from the group consisting of D, Q, Y and N,

HX₉ is selected from the group consisting of N, Q, S and I.

6. A specific binding member according to any one of claims 1
15 to 5 that neutralizes human IL-13.

7. A specific binding member according to claim 6 that
neutralizes human IL-13, with a potency equal to or better
than the potency of a IL-13 antigen-binding site formed by the
20 BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain
(SEQ ID NO: 16), the potency of the specific binding member
and the potency of the antigen-binding site being as
determined under the same conditions.

25 8. An isolated specific binding member according to any one
of claims 1 to 7 which binds a human IL-13 variant in which
arginine at position 130 is replaced by glutamine.

9. An isolated specific binding member according to any one
30 of claims 1 to 7 which binds non-human primate IL-13.

10. An isolated specific binding member for human IL-13,
comprising an antibody antigen-binding site which is composed
of a human antibody VH domain and a human antibody VL domain

and which comprises a set of CDR's HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the set of CDR's consists of a set of CDR's selected 5 from the group consisting of:

the BAK278D6 set of CDR's, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid 10 sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6,

a set of CDR's which contains one or two amino acid substitutions compared with the BAK278D6 set of CDR's, and 15 each set of CDR's as shown for individual clones in Table 1.

11. An isolated specific binding member according to claim 10 wherein the one or two substitutions are at one or two of the 20 following residues within the CDRs, using the standard numbering of Kabat.

31, 32, 34 in HCDR1

25 52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2

96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

30

56 in LCDR2

95A, 97 in LCDR3

12. An isolated specific binding member according to claim 11 wherein the one or two substitutions are made at the following positions from among the identified groups of possible substitute residues for each position:

5

<u>Position of substitution</u>	<u>Substitute Residue selected from the group consisting of</u>
---------------------------------	---

10 31 in HCDR1: Q, D, L, G and E

32 in HCDR1: T

34 in HCDR1: V, I and F

15 52 in HCDR2: D, N, A, R, G and E

52A in HCDR2: D, G, T, P, N and Y

53 in HCDR2: D, L, A, P, T, S, I and R

54 in HCDR2: S, T, D, G, K and I

56 in HCDR2: T, E, Q, L, Y, N, V, A, M and G

20 58 in HCDR2: I, L, Q, S, M, H, D and K

60 in HCDR2: R

61 in HCDR2: R

62 in HCDR2: K and G

64 in HCDR2: R

25 65 in HCDR2: K

96 in HCDR3: R and D

97 in HCDR3: N, D, T and P

98 in HCDR3: R

30 99 in HCDR3: S, A, I, R, P and K

101 in HCDR3: Y

26 in LCDR1: D and S

27 in LCDR1: I, L, M, C, V, K, Y, F, R, T, S, A, H and G

28 in LCDR1: V

30 in LCDR1: G

31 in LCDR1: R

5 56 in LCDR2: T

95A in LCDR3: N

97 in LCDR3: I.

10 13. An isolated specific binding member according to claim 12 wherein there are two substitutions compared with the BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue 27.

15 14. An isolated specific binding member according to claim 13 comprising the BAK278D6 set of CDR's with a substitution at HCDR3 residue 99 selected from the group consisting of S, A, I, R, P and K, and/or a substitution at LCDR1 residue 27 selected from the group consisting of I, L, M, C, V, K, Y, F, R, T, S, A, H and G.

20 15. An isolated specific binding member according to claim 13 comprising the BAK278D6 set of CDR's with S substituted for N at HCDR3 residue 99 and/or I substituted for N at LCDR 1 residue 27.

25 16. An isolated specific binding member according to any one of claims 10 to 15 wherein HCDR1, HCDR2 and HCDR3 of the VH domain are within a germ-line framework and/or LCDR1, LCDR2 and LCDR3 of the VL domain are within a germ-line framework.

30 17. An isolated specific binding member according to any one of claims 10 to 16 which binds a human IL-13 variant in which arginine at position 130 is replaced by glutamine.

18. An isolated specific binding member according to any one of claims 10 to 17 which binds non-human primate IL-13.

19. A specific binding member according to any one of claims 5 16 to 18 comprising the BAK502G9 VH domain (SEQ ID NO: 15).

20. A specific binding member according to any one of claims 16 to 19 comprising the BAK502G9 VL domain (SEQ ID NO: 16).

10 21. A specific binding member according to any one of claims 10 to 20 that binds IL-13 with affinity equal to or better than the affinity of an IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain (SEQ ID NO: 16), the affinity of the specific binding 15 member and the affinity of the antigen-binding site being as determined under the same conditions.

22. A specific binding member according to any one of claims 10 to 21 that neutralizes human IL-13.

20 23. A specific binding member according to claim 22 that neutralizes human IL-13, with a potency equal to or better than the potency of a IL-13 antigen-binding site formed by the BAK502G9 VH domain (SEQ ID NO: 15) and the BAK502G9 VL domain 25 (SEQ ID NO: 16), the potency of the specific binding member and the potency of the antigen-binding site being as determined under the same conditions.

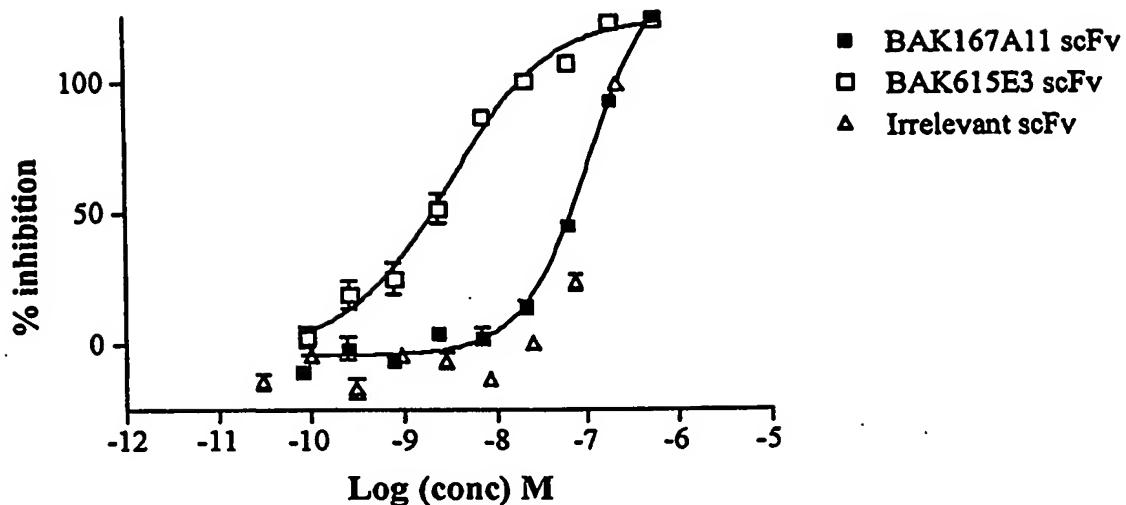
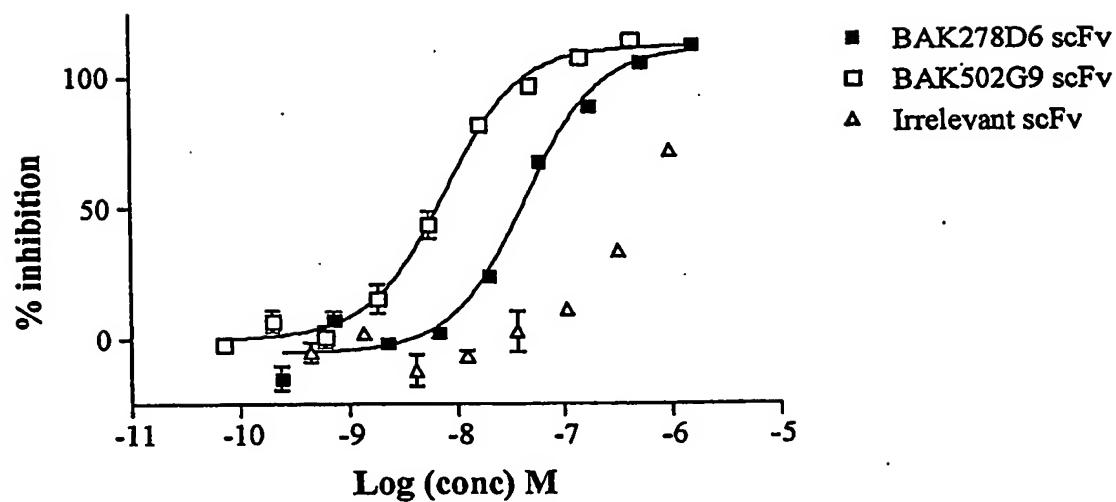
Figure 1**Figure 2**

Figure 3

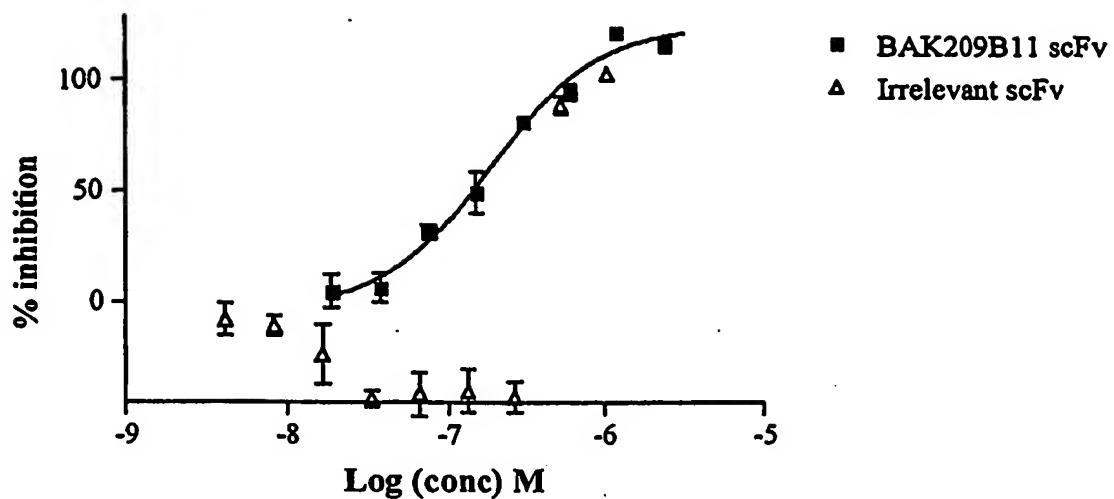


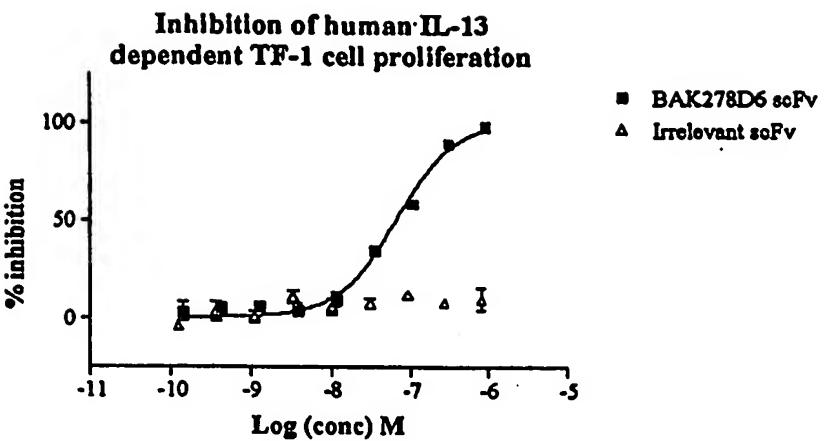
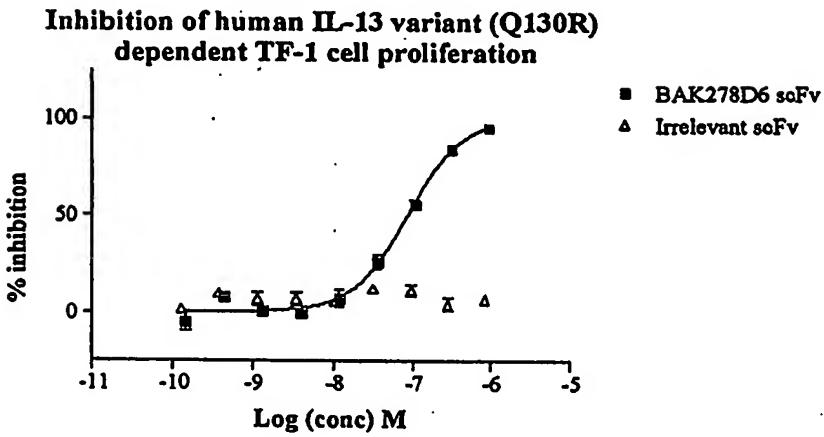
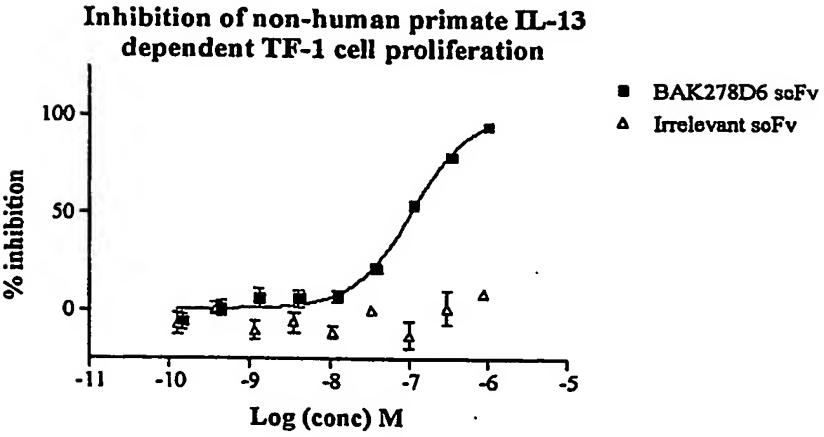
Figure 4A**Figure 4B****Figure 4C**

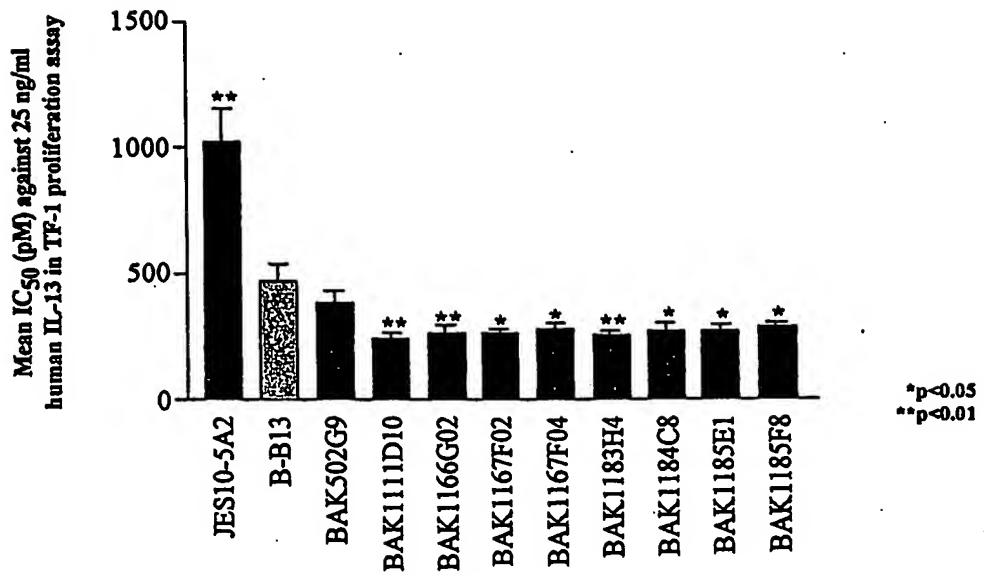
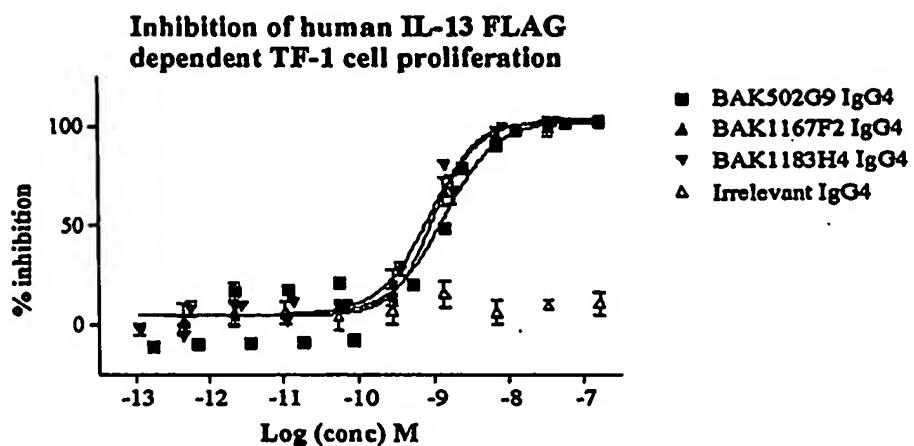
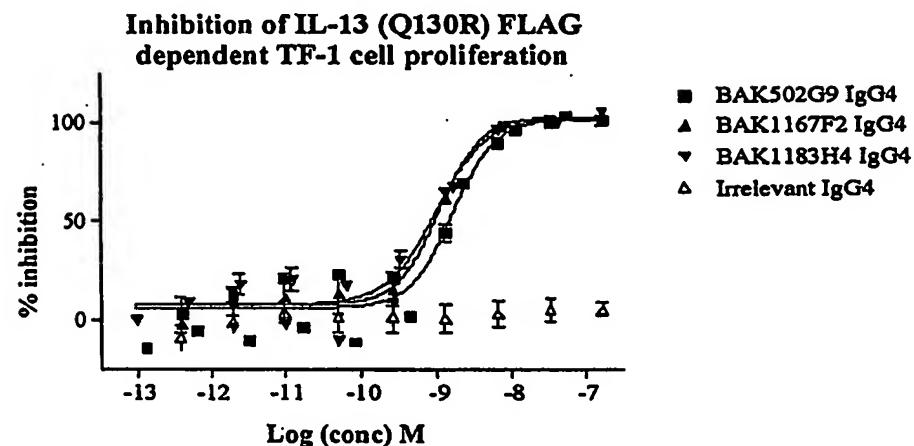
Figure 5

Figure 6A**Figure 6B****Figure 6C****Inhibition of non-human primate IL-13 (FLAG-tagged) dependent TF-1 cell proliferation**

% inhibition

Log (conc) M

- BAK502G9 IgG4
- ▲ BAK1167F2 IgG4
- ▼ BAK1183H4 IgG4
- △ Irrelevant IgG4

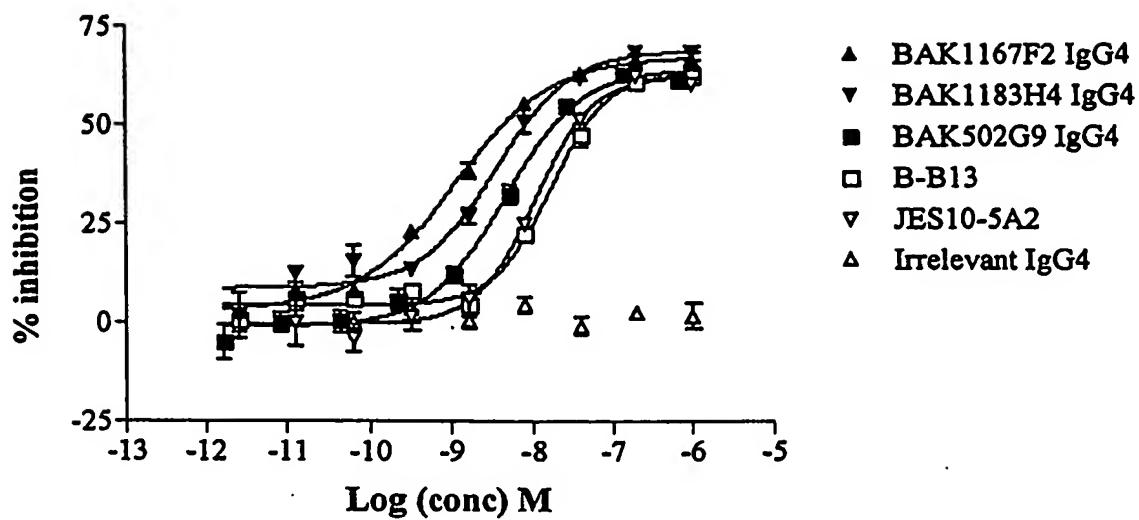
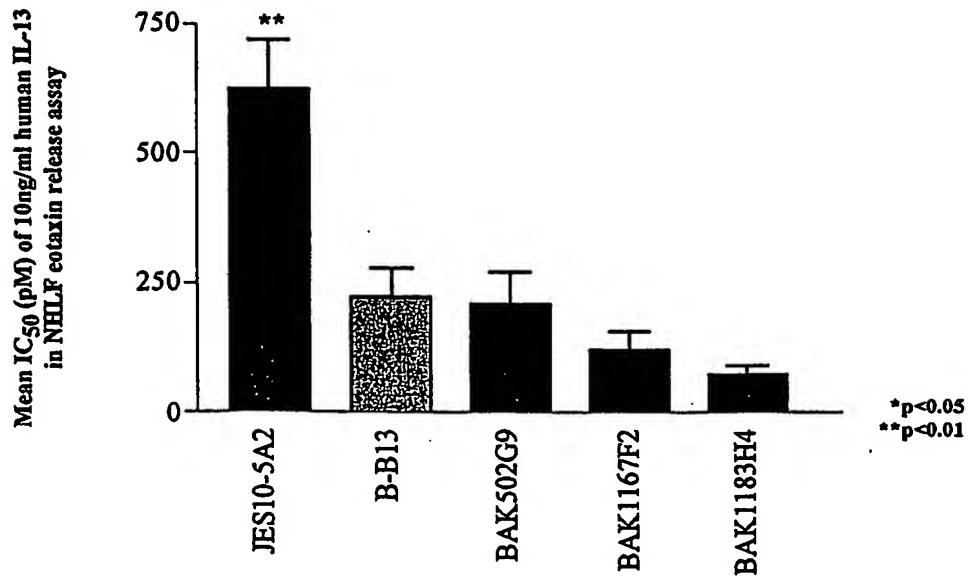
Figure 7**Figure 8**

Figure 9

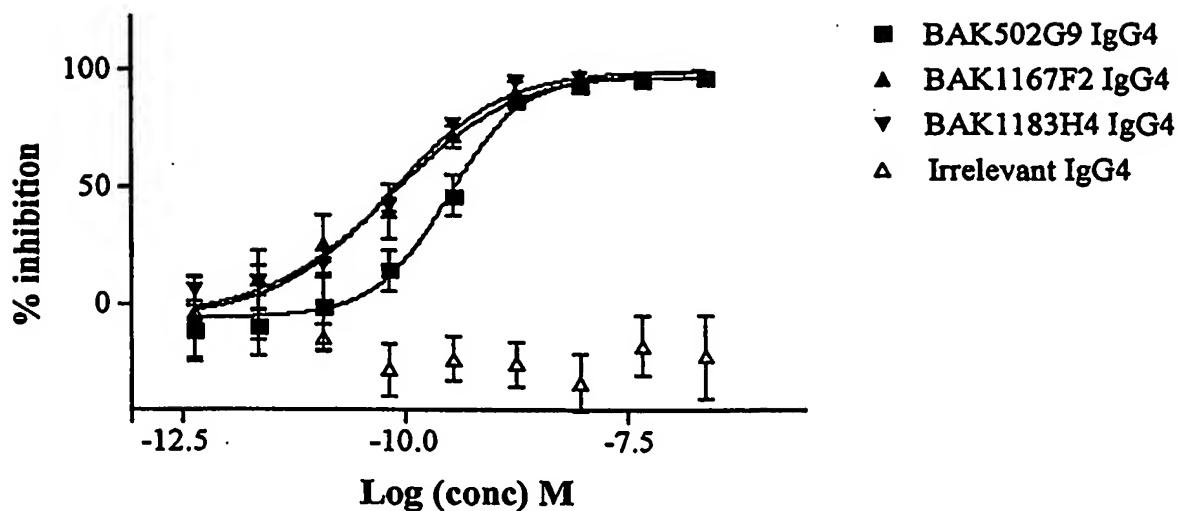


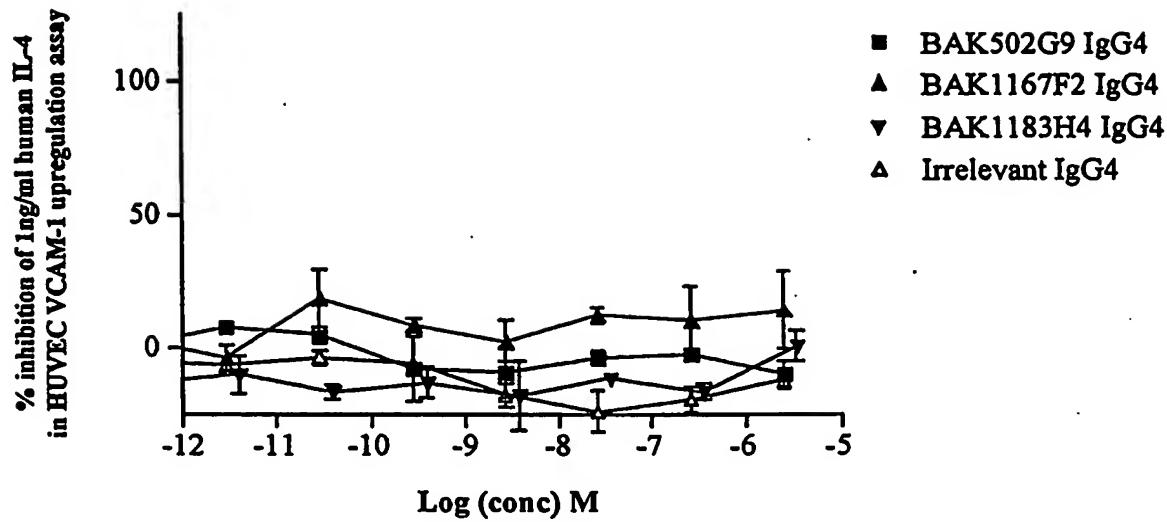
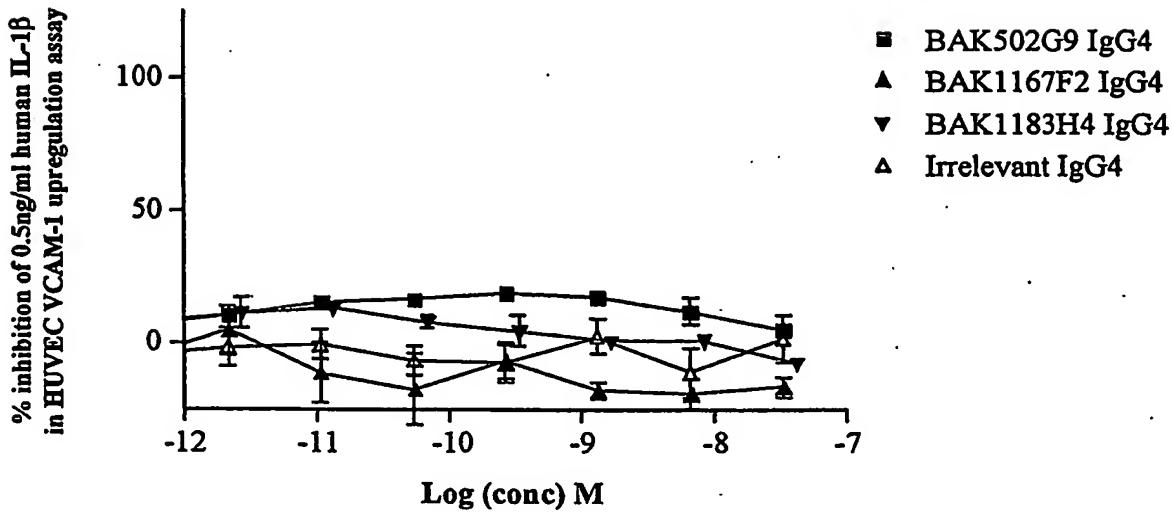
Figure 10A**Figure 10B**

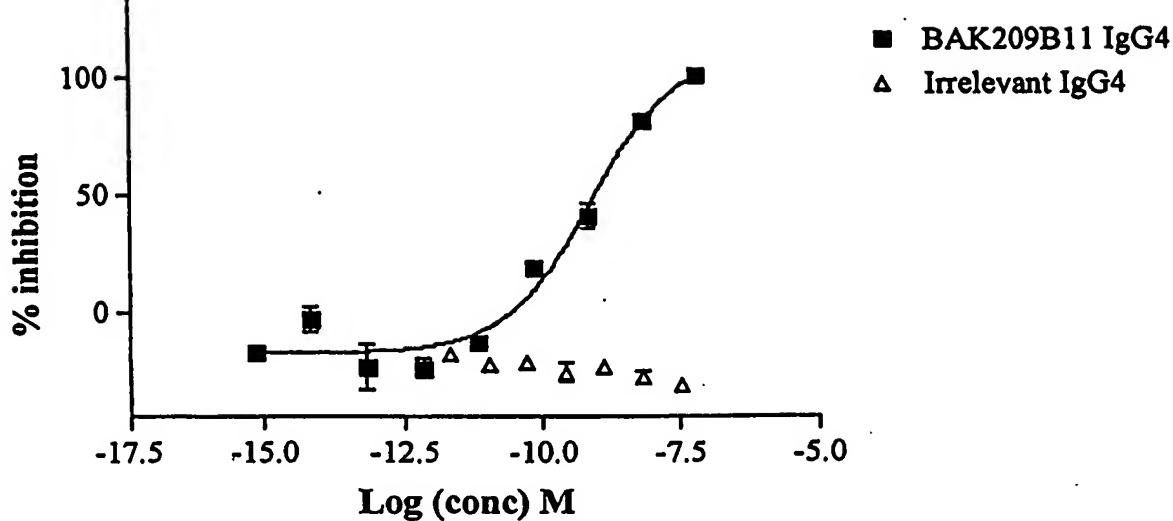
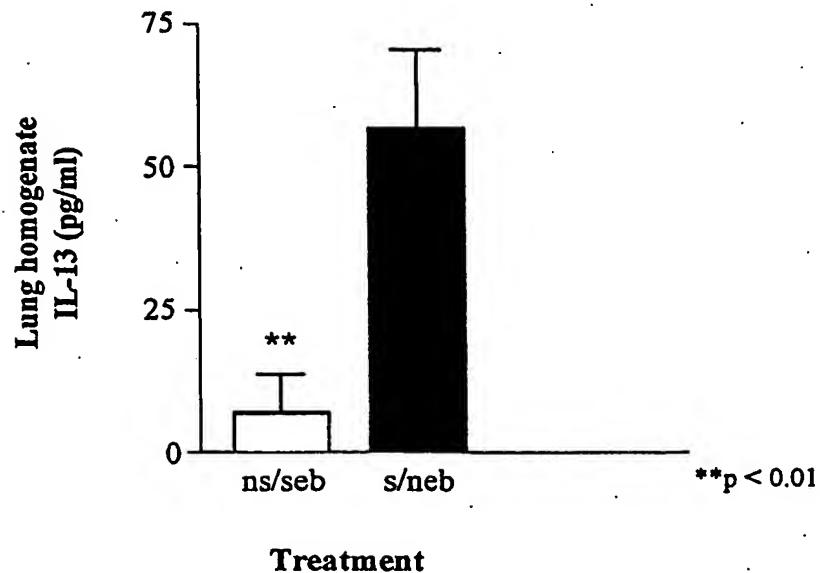
Figure 11**Figure 12**

Figure 13

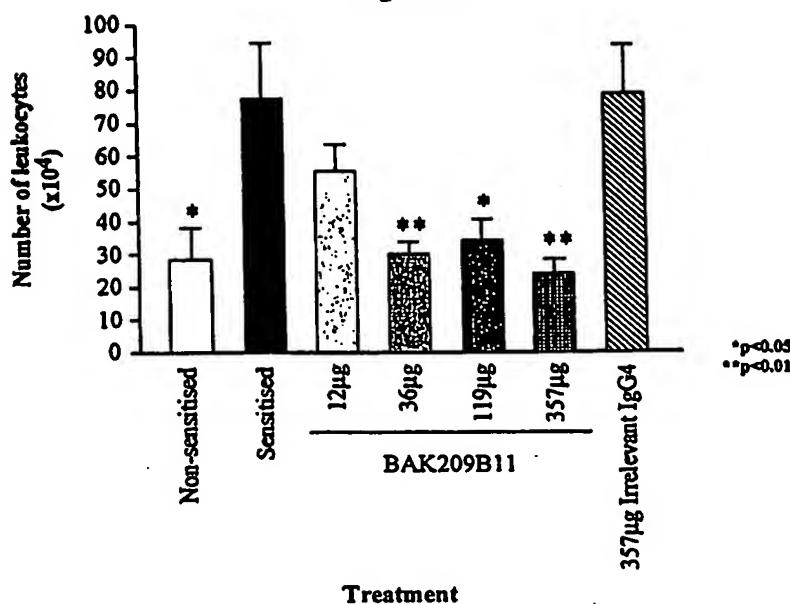


Figure 14

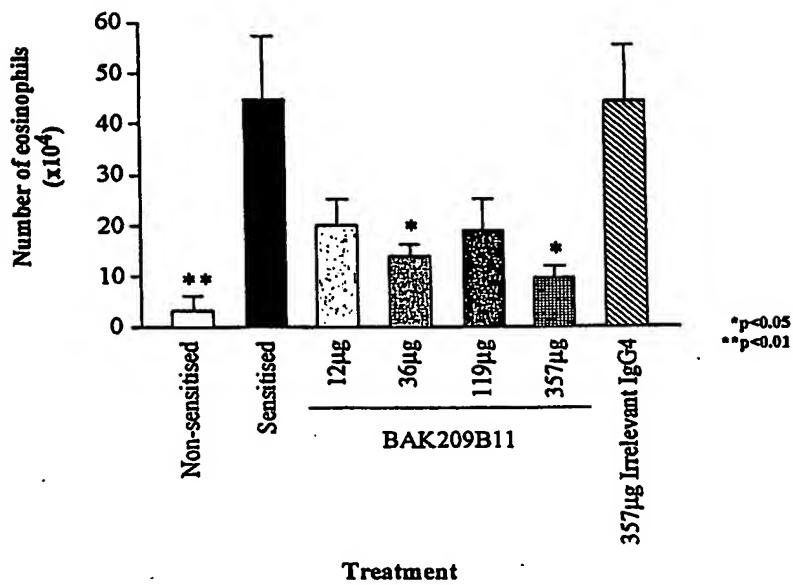


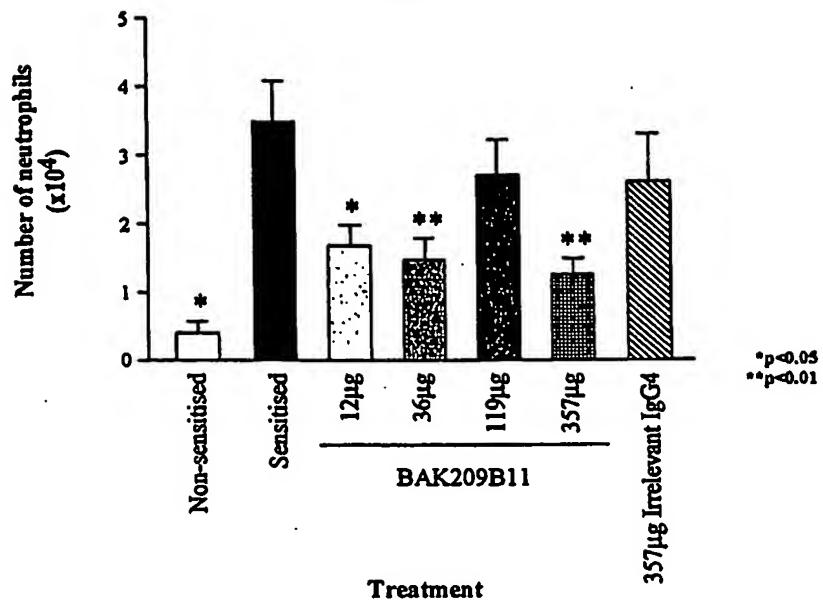
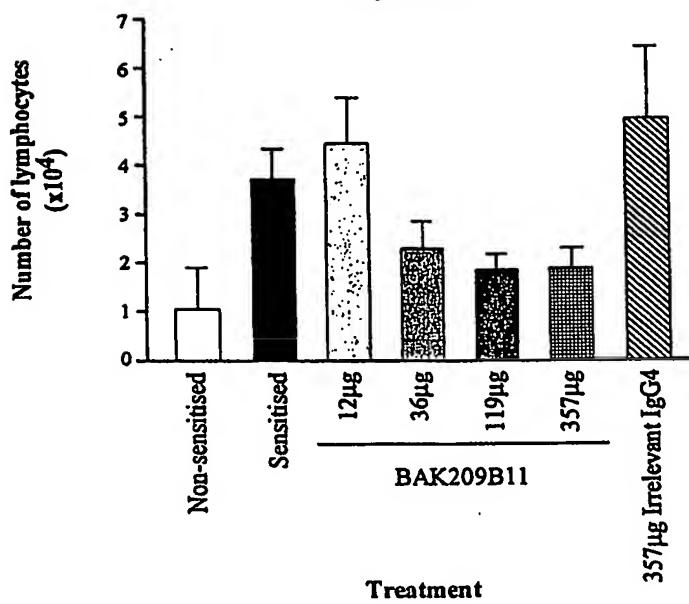
Figure 15**Figure 16**

Figure 17

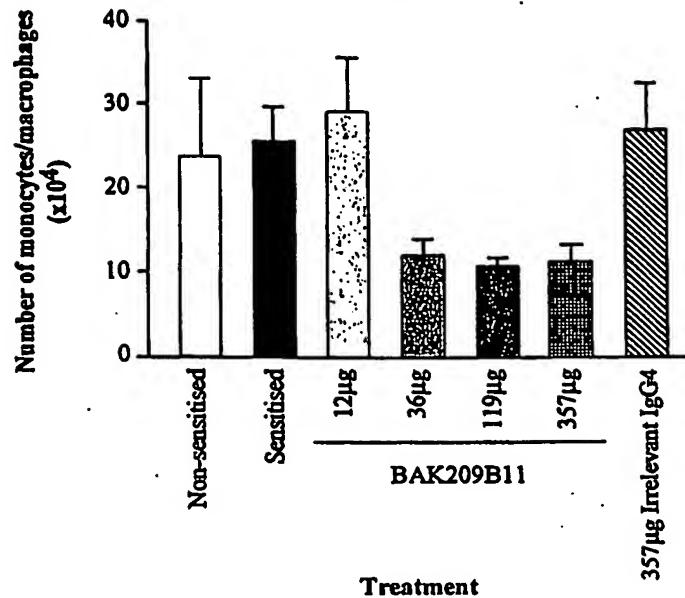


Figure 18

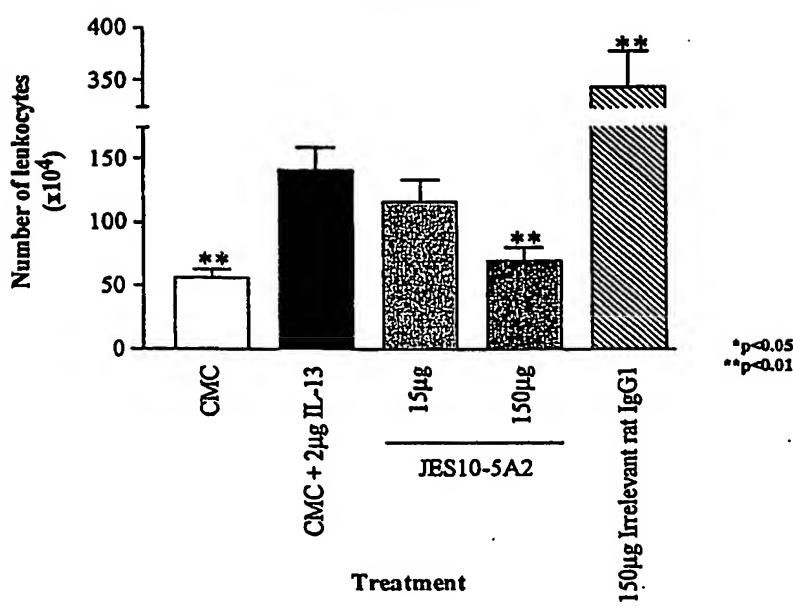


Figure 19

<i>Human IL-13</i>	MALLLT	TVIALT	CLGGFASP	P	PVPP	STAL	R ELIEELVNIT	30	40
<i>Cynomolgus IL-13</i>	MALLLT	TVIALT	CLGGFASP	S	PVPP	STAL	K ELIEELVNIT		
	MALLLT	TVIALT	CLGGFASP		PVPP	STAL	ELIEELVNIT		
<i>Human IL-13</i>	QNQKAP	LCNGSMVWSINLTAG	M YCAALESLINVGCSAIE	50	60	70	80		
<i>Cynomolgus IL-13</i>	QNQKAP	LCNGSMVWSINLTAG	V YCAALESLINVGCSAIE						
	QNQKAP	LCNGSMVWSINLTAG	YCAALESLINVGCSAIE						
<i>Human IL-13</i>	KTQRML	S GFCPHKVSAGQFSSL	H VRDTKIEVAQFVKDLL	90	100	110	120		
<i>Cynomolgus IL-13</i>	KTQRML	N GFCPHKVSAGQFSSL	R VRDTKIEVAQFVKDLL						
	KTQRML	GFCPHKVSAGQFSSL	VRDTKIEVAQFVKDLL						
<i>Human IL-13</i>	HLKKL	FREG	R FN	130					
<i>Cynomolgus IL-13</i>	HLKKL	FREG	Q FN						
	HLKKL	FREG	FN						

Figure 20

Effect of a single 10mg/kg dose of BAK502G9 (IgG4) on serum IgE levels in allergic but non-challenged cynomolgus monkeys

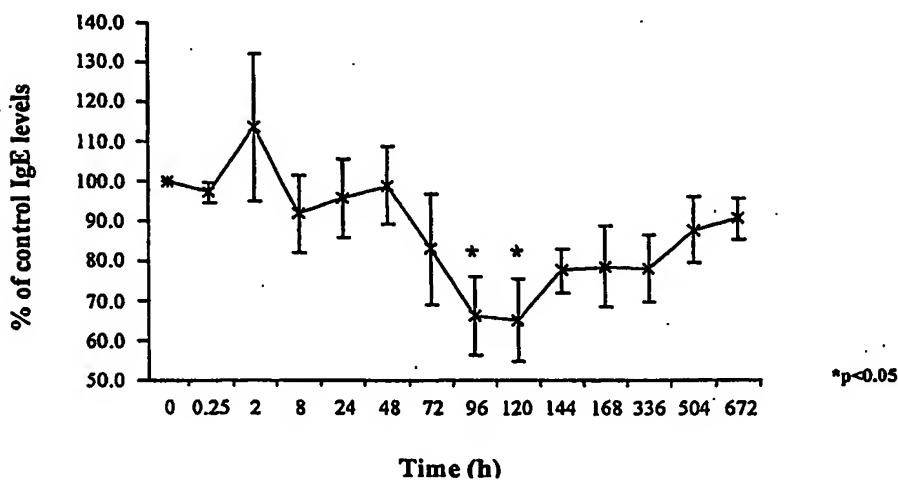
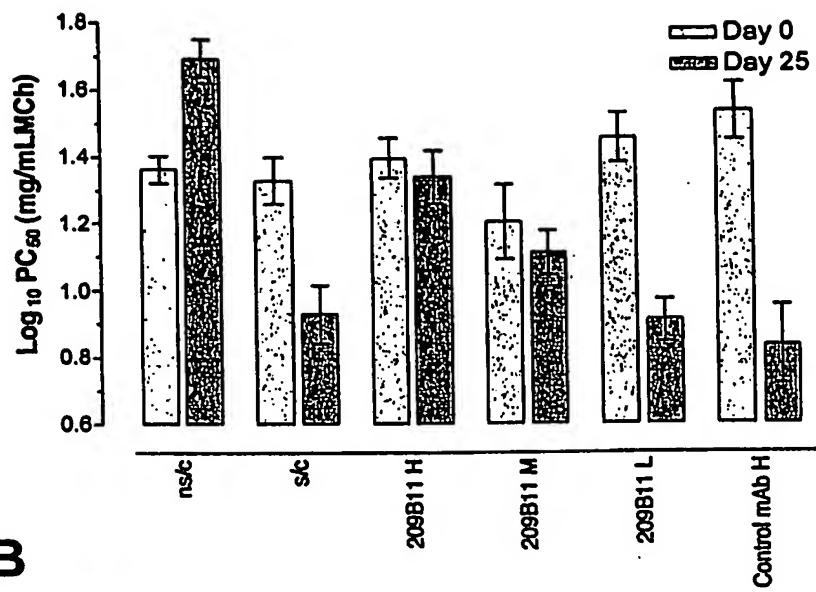
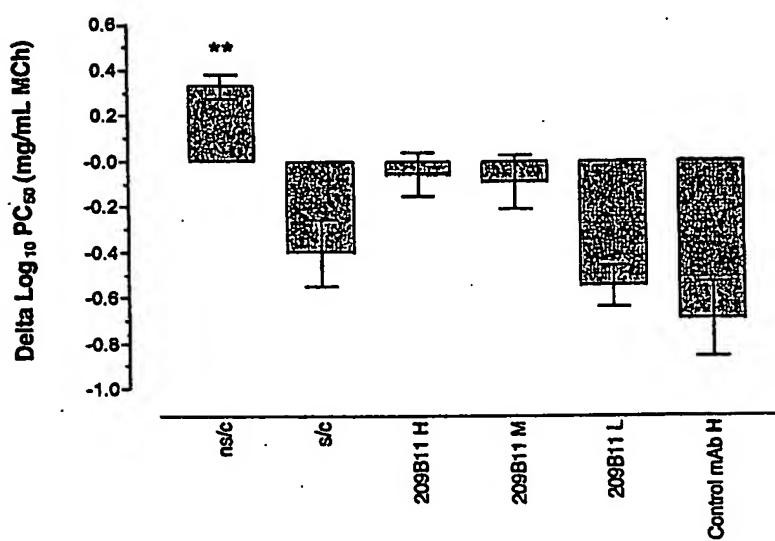


Figure 21

A



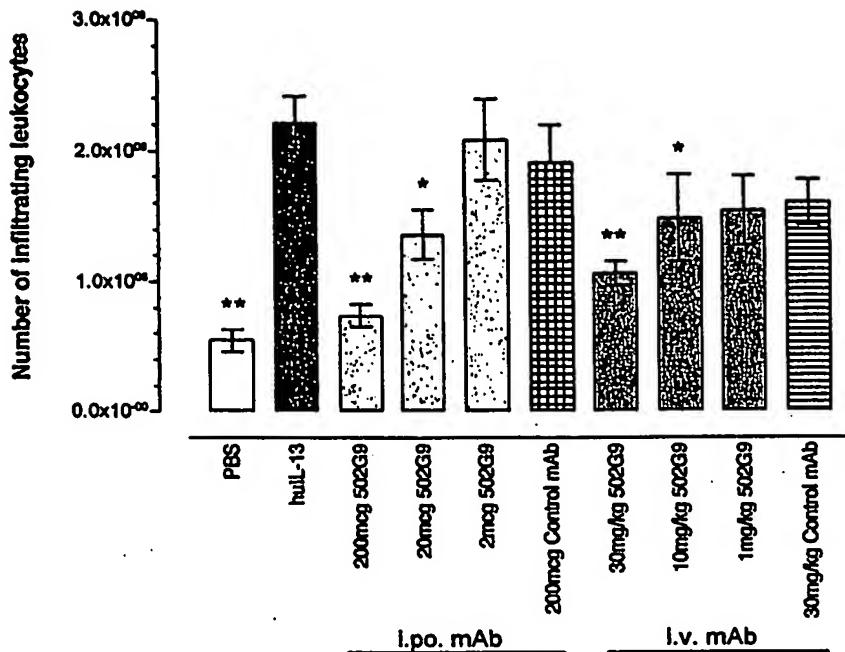
B



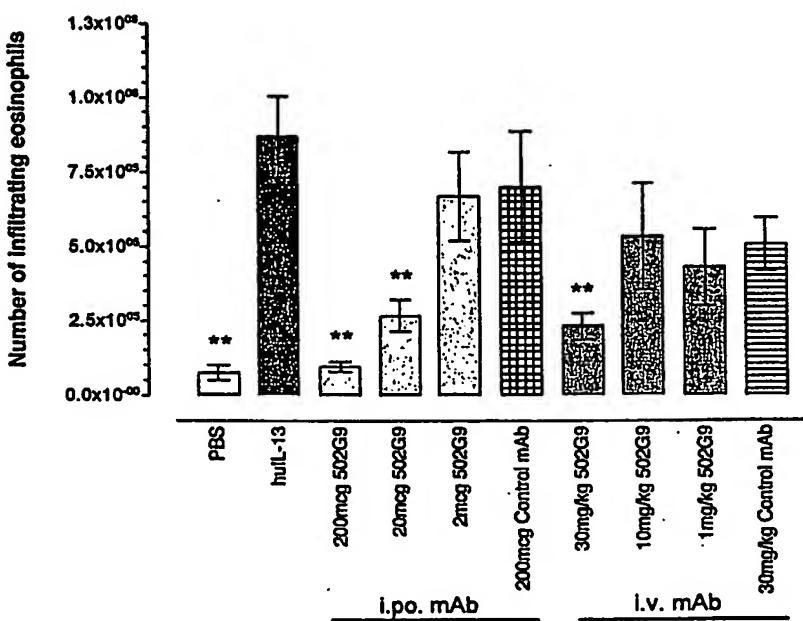
** p<0.01 vs s/c control; One-way ANOVA followed by
Dunnett's multiple comparisons test

Figure 22

A



B



A and B; *p<0.05, **p<0.01 vs huIL-13 control; One-way ANOVA on log-transformed data, followed by Dunnett's multiple comparisons test.